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THE UNIVERSITY OF ALBERTA

REPAIR PARAMETERS IN MUTATOR MUTANTS OF *SACCHAROMYCES CEREVISIAE*

BY

© DONALD PATERSON MORRISON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

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ABSTRACT

The effects of a series of mutator mutants on UV-induced mutation and UV-induced intra- and intergenic recombination, parameters associated with the DNA repair system of yeast, were assayed to establish a clearer understanding of the relationship between the mutator loci and DNA repair.

No marked effect of *mut1-1*, *mut2-1*, *mut3-1*, *mut4-1*, *mut6-1* or *mut9-1* on any of these parameters was apparent. Homozygous *mut5-1* diploid strains were found to be deficient in UV-induced intragenic recombination at *his 1*. Mutation is sufficient to account for all the UV-induced histidine prototrophs in *mut5-1/mut5-1*, heteroallelic *his 1* diploids.

While no effect of *mut5-1* on the frequency of UV-induced homozygosis of *ade2-1* was apparent, homozygous and heterozygous *mut5-1* diploids produce, at higher frequency than the wild type, spontaneous or UV-induced segregants in which heterozygous recessive markers on both arms of linkage group V were uncovered simultaneously. The viability of 3 or 4 spores/tetrad for most asci dissected from aberrant segregants of a heterozygote is not consistent with these segregants being the result of a single non-disjunction event. Two such events, or two crossovers, one on each side of the centromere involving the same two chromatids, are required.

The frequencies of intragenic recombination at *his 1* and intergenic recombination between *hom 3* and *arg 6* in the infrequent viable meiotic products obtained from *mut5-1* homozygous diploids were found to be no different from wild type or heterozygous strains. The failure of *mut5-1/mut5-1* diploid strains to exhibit wild type frequencies of intragenic recombinants on removal from sporulation medium to selective nutrient medium indicates an inability to establish and/or resolve a meiotic precondition.

Allelism of *mut5-1* and *rad 51-1* is indicated by their failure to complement to restore radioresistance and efficient sporulation, the close linkage of both to *trp 2* and their very similar phenotypes.

The effect of mating-type genotype on expression of the mutator phenotype was also investigated. Homozygous *mut3-1* or *mut4-1*, *a*/α diploids exhibit reduced mutator activity. Restoration of the mutator phenotype is observed when mating-type is rendered homozygous. In diploids homozygous for *mut10-1* this relationship is reversed. It is suggested that in homozygous *mut3-1* or *mut4-1*, *a*/α diploid strains, spontaneous lesions, which may be processed mutagenically in haploid or homozygous mating-type diploids, are resolved preferentially and non-mutagenically by an *a*/α-dependent process; and further, that *MUT 10* encodes a component of this system. A block at the *MUT 10* step results in redirection of the lesions from the non-mutagenic process to a mutagenic one.

That the *a*/α-effect on spontaneous mutation is of the same origin as that described by Laskowski (1962) for X-ray inactivation is suggested by the failure of *mut5-1/mut5-1*, *a*/α diploids to exhibit reduced mutator activity or increased γ-ray resistance relative to their *a*/*a* counterparts.

No *a*/α-effect on spontaneous mutation was seen in strains homozygous for *mut1-1*, *mut2-1*, *mut6-1* or *mut9-1*. The absence of pronounced X-ray sensitivity of strains carrying these mutations makes it unlikely that they are components of the *a*/α-dependent system. The extent of their interaction with the DNA repair system of yeast remains unclear.

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INTRODUCTION

That changes in spontaneous mutation rates can be effected by genetic manipulation has been clearly demonstrated in bacteriophage T4 (reviewed by Drake, 1973), in the bacteria *Escherichia coli*, *Salmonella typhimurium* and *Bacillus subtilis* (reviewed by Cox, 1976), in the fungi *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Aspergillus nidulans* (von Borstel et al, 1973; Loprieno, 1973; and Jansen, 1972), and in Drosophila (reviewed by Green, 1973). However, with few exceptions, notably the gene *43* mutants of T4 which will be discussed at length later, the mechanisms of spontaneous mutation are not understood.

The purpose of the work to be described here was to expand the characterization of some mutator mutants of *S. cerevisiae* in the hope that this would bring us closer to understanding the nature of their normal functions. The review of spontaneous mutation in T4 and *E. coli* which follows is designed to describe the context in which the work was done, and to justify to some extent the approach that was taken.

Several hypotheses have been proposed to explain how spontaneous mutation and the mutator phenotype may arise. Tautomeric base changes resulting in incorporation errors at replication were suggested as the basis for spontaneous mutation by Watson and Crick (1953). More recently, Topal and Fresco (1976), extended the scope of such changes by invoking the occurrence of nucleotide configurations not previously considered.

Kirchner (1960) offered the possibility of endogenous production and incorporation into DNA of a base analogue as an explanation of the mode of action of a mutator in *S. typhimurium*. A deficiency of a DNA precursor, as a consequence of mutation in a gene encoding an enzyme

involved in its biosynthesis, could result in an increased spontaneous mutation rate by misincorporation of other nucleotides in its stead (Liberfarb and Bryson, 1970). The observation that mutants of T4 deficient in hydroxymethyl-dCTP, and TTP biosynthesis are also mutators (Drake, 1973) seems to support this hypothesis.

The proposal that reduced DNA repair capabilities could result in an increase in spontaneous mutation rate due to the inability of repair mutants to process accidentally misincorporated bases after replication, was presented by Hanawalt and Haynes (1965). Such defects would also be manifested in the sensitivity of the mutants to agents known to interact with DNA.

The hypothesis that the rate of spontaneous mutation was governed by the "proof-reading/editing", 3'→5' exonuclease moiety of the T4 and *E. coli* DNA polymerases during replication was proposed by Goulian et al, 1968.

Spontaneous Mutation in T4

The view that spontaneous mutations are the result of misincorporation of nucleotides in the course of DNA replication gained considerable strength from the work of Muzyczka et al, (1972) on DNA polymerases purified from gene *43 ts* mutants of T4. Gene *43* encodes a single polypeptide having two enzymatic functions, 5'→3' DNA polymerase and 3'→5' exonuclease (Goulian et al, 1968). Temperature sensitive (ts) mutations of gene *43* have been isolated which confer mutator, antimutator or neutral (like wild type) phenotypes at the permissive temperature on strains carrying them (Speyer et al, 1966; Drake and Allen, 1968; Drake et al, 1969). *In vitro* assays of polymeric and exonucleolytic activities of DNA polymerases purified from such strains showed that mutators have

lower ratios of exonucleolytic to polymeric activities than wild type, and that in antimutators the exonucleolytic to polymeric ratios are higher. In the neutral mutants the ratios were like the wild type. In antimutators increased nucleotide pool turnover was also observed, consistant with the increased editing prediction. Spontaneous mutation rates, it was proposed, are determined by the relative rates of insertion and removal of nucleotides by the polymerase/exonuclease during replication. It has since been confirmed that, *in vitro*, mutator polymerases incorporate incorrect nucleotides more often, and antimutator polymerases less often, than the wild type (Hershfield and Nossal, 1973; Schnaar et al, 1973).

Spontaneous Mutation and the DNA Polymerases of *E. coli*

All three of the DNA polymerases identified in *E. coli*, pol I, pol II and pol III have been shown to possess 3'→5' exonucleolytic capability (see Kornberg, 1974) and to remove mismatched terminal nucleotides in *in vitro* systems--that is, they have editing capacity (Brutlag and Kornberg, 1972; Smith et al, 1976). As yet, however, deficiencies in this activity have not been correlated with mutator activity for any of them.

In addition to its 3'→5' exonuclease activity, pol I exhibits 5'→3' polymerase and 5'→3' exonuclease functions (Kornberg, 1974). Mutator activity has been observed in mutants deficient in either of these functions. A knowledge of some additional properties of *pol A* mutants, makes it possible to provide a rationalization, albeit hypothetical, for their mutator phenotypes that does not rely on misincorporation during scheduled DNA replication.

Pol I is involved in the joining of Okazaki fragments during replication (Okazaki et al, 1971)--the polymerase filling the gap created by the 5'→3' exonuclease as it degrades the RNA primer (Kornberg, 1974). In *pol A*$^-$ mutants, the gaps between the 3'-ends of newly synthesized fragments and the 5'-ends of the RNA primers are relatively long-lived. That the processing of these gaps in *pol A*$^-$ strains is taken over, at least in part, by the functions encoded by *rec A*, *rec B* and *rec C*, can be inferred from the observations that the combination of *pol A*$^-$ with *rec A*$^-$, *rec B*$^-$ or *rec C*$^-$ is lethal (Gross et al, 1971; Monk and Kinross, 1972; Smirnov et al, 1973). The involvement of these three loci in recombination (Ogawa et al, 1968) and the increased frequencies of recombination seen in *pol A*$^-$ mutants (Konrad and Lehman, 1974) tends to support this.

Rec A, however, is also essential for the function of the inducible SOS repair system of *E. coli* (see the review by Witkin, 1976). This system, which is mutagenic, appears to be activated by, among other things, the persistence of gaps in DNA (Witkin, 1976). It is clearly possible that the increased spontaneous mutation rates seen in *pol A* mutants which are slow to join Okazaki fragments could result from the intermittent activation of the SOS repair system by the slowly resolved gaps that occur in such strains at replication.

Mutation of the *dnaE* gene of *E. coli*, which encodes DNA polymerase III, can also result in mutator activity. Seventeen of twenty mutants, identified initially on the basis of temperature-sensitive DNA synthesis (Wechsler and Gross, 1971; Sevastopoulos and Glaser, 1977), have been shown to be mutators (Hall and Brammer, 1973; Sevastopoulos et al, 1977). As yet, nothing is known of their mode of action.

No mutant alleles of *pol B*, the polymerase II gene, have been shown to produce the mutator phenotype.

Other Mutator Loci of *E. coli*

Mutation of the *mut U* (*uvrE*) gene of *E. coli* results in UV-sensitivity and mutator activity (Smirnov et al, 1972; Horiuchi and Nagata, 1973; Siegel, 1973). Three independently isolated alleles are lethal in combination with temperature-sensitive *pol A* mutations (Horiuchi and Nagata, 1973; Siegel, 1973; Smirnov et al, 1973), suggesting that, like *rec A, B* or *C*, the *mut U* encoded function may replace a pol I function during replication. In the absence of any information on the precise role of *mut U*, further speculation is inadvisable.

E. coli K12 strains carrying mutations in the *dam* gene were identified on the basis of undermethylation of their DNA (Marinus and Morris, 1973). They are also UV- and mitomycin C-sensitive, and exhibit increased spontaneous mutability (Marinus and Morris, 1974). The data presented in the latter paper lead to the conclusion that the undermethylation of the *dam 3* strains DNA leads to nucleolytic restriction, the resulting gaps being subject to repair processing involving polymerase I, ligase and/or the *rec A*, *rec B* and *rec C* gene products. As for the *pol A*$^-$ strains, the mutator activity observed in *dam*$^-$ strains is explicable as a consequence of the processing of the restriction nuclease lesions by SOS repair.

Five loci in *E. coli* have been identified as mutator genes strictly on the basis that mutations at them result in mutator phenotype, *mut T* (Treffers et al, 1954), *mut S* (Siegel and Bryson, 1964), *mut L* (Liberfarb

and Bryson, 1970), *mut D* (Degnen and Cox, 1974), and *mut R* (Hoess and Herman, 1975).

Mutant alleles of *mut T* have been shown to result in an increase of spontaneous A:T → C:G transversions (Yanofsky et al, 1966; Cox, 1973; Conrad et al, 1974), other mutation types occurring apparently at wild type rates. That the expression of the mutator phenotype may be dependent on DNA synthesis has been shown in experiments involving density-labelled phage λ (Cox, 1970). Unreplicated phage retrieved from *mut T1* cells exhibit little mutation. Phage containing once or twice replicated DNA show markedly increased mutation frequencies. A further indication that *mut T1* may be involved in replication is that it interacts with *dnaE293* (pol III defective) to relieve partially the temperature-sensitive DNA synthesis associated with *dnaE293* (Cox, 1973).

mut S mutations are recessive and active in *trans* on *F'lac* (Cox et al, 1972). The spontaneous mutations which occur in *mut S*⁻ strains are believed to be of the transition (Cox et al, 1972) and frameshift (Siegel and Kamel, 1974) types.

mut L is located on the *E. coli* genetic map (Siegel and Ivers, 1975) in a homologous position to the mutator gene in strain LT7 of *S. typhimurium* (Kirchner, 1960), whose cotransducibility with *pur A* led Kirchner to propose his endogenous mutagen hypothesis. Siegel and Ivers (1975) have argued that the failure of exogenous adenine to suppress the mutator phenotype, and the retention of mutator activity in *mut L25 pur A53* double mutants (in *E. coli*) indicates that *mut L25* is not an allele of *pur A*. Several other mutator mutations have been mapped to this region, their cotransducibilities with *pur A* ranging from 80 to 100% (Liberfarb and Bryson, 1970). *mut L25* is active in *trans* on an F-episome, and is believed to induce A:T → G:C and G:C → A:T transitions (Siegel and Ivers,

1975). That it does not induce transversions was not established. This mutator had been shown previously to induce frameshift reversions (Siegel and Kamel, 1974).

Two alleles of *mut D* have been isolated (Degnen and Cox, 1974). Strains carrying either exhibit mutant frequencies 10-100 times that of the wild type after overnight growth in minimal medium, and 10^3-10^5 times the wild type frequency after overnight growth in broth (Degnen and Cox, 1974). Three "effectors" have been identified, thymidine (Degnen and Cox, 1974), deoxyuridine and deoxycytidine (Ehrlich and Cox, 1974). That they must be phosphorylated to become potent is indicated by the failure of thymidine kinase mutants to respond (Cox, 1976). Cox favours the view that the *mut D* product functions during replication *per se*, rather than in recombination or repair, because *mut D5* related mutator activity is not observed in the absence of DNA synthesis, and is seen in a *rec A*$^-$ background (Degnen, cited by Cox, 1976). The spontaneous mutations produced are believed to be of all types--transitions, transversions and frameshifts (Fowler et al, 1974).

The mutator *mut R* has been shown to increase the rate of frameshift reversions and base substitutions, to be recessive, to act on *F'lac* in *trans*, to be viable in combination with *pol A*$^-$, *pol B*$^-$ *lig*$^-$ and *uvr A*$^-$ markers and to increase recombination (Hoess and Herman, 1975).

It is apparent that the editing efficiency mechanism which has been so clearly defined with T4 DNA polymerases *in vitro* is not a sufficient explanation for the *E. coli* system. With the exception of *pol A* and *dam*, the data that has been accumulated on the effect of the mutators does little to explain how they give rise to the mutator phenotype. It is significant, I think, that the most useful data obtained were either biochemical, or based on the interaction of the mutator loci with mutants

in repair processes whose functions, hypothetically at least, are understood.

Understanding Mutators in Yeast

The argument has been made (Hastings et al, 1976) that the editing efficiency hypothesis is inadequate as an explanation of mutator mutants in *S. cerevisiae*, insofar as more loci (8 definite and 4 probable in an unsaturated system) have been identified than would appear to be necessary. Their observations, which will be discussed later, led them to conclude that spontaneous mutation may also result from the processing of spontaneous lesions by mutagenic repair pathways (cf. Hanawalt and Haynes, 1965 and Witkin, 1976), independent of the replication schedule.

In this context, and in view of the *E. coli* results, it is clear that some insights into the normal function of mutators in yeast may be derived from an understanding of where mutators fit in the overall scheme of repair systems in yeast as they are currently defined.

DNA Repair in Yeast

Radiation repair pathways in *S. cerevisiae* have been defined primarily on the basis of the interaction of mutant genes apparent in the survival of double mutants following UV exposure (see Cox and Game, 1974). Three interactions have been described, epistatic, additive and synergistic. Epistasis describes the observation that the double mutant is no more sensitive than the more sensitive of the single mutants from which it is derived, and is taken to mean that the gene products identified by the mutants function in the same repair pathway. When the surviving fraction of the double mutant over a range of doses approximates the product of the surviving fractions of the single

mutants over the same range, additivity holds. In the case of non-leaky mutants, this interaction is used to place the genes on independent pathways at points from which no further processing of their substrates is possible. The third interaction, synergism, describes situations in which the double mutant is more sensitive than is predicted for an additive interaction. Such interactions are the expectation if the mutations affect two different repair routes and one of them causes a block at the first step of its pathway.

On the basis of these interactions three pathways have been described: the first involves *RAD 1*, *RAD 2*, *RAD 3*, *RAD 4* and *RAD 22* (Nakai and Matsumoto 1967; Game and Cox, 1972; Brendel and Haynes, 1973: Lawrence and Christensen, 1976); the second, *RAD 6*, *RAD 18*, *REV 1*, *REV 2* and *REV 3* (Lemontt, 1971a; Lawrence et al, 1974; Lawrence, cited by Haynes, 1975) and the third *RAD 50* and *RAD 51* (Cox and Game, 1974). For convenience, in the following discussion, the three pathways will be referred to as the *RAD 3*, and *RAD 18* and the *RAD 51* pathways, for the first step in each as defined by synergistic interactions (Nakai and Matsumoto, 1967; Game and Cox, 1973; Cox and Game, 1974).

The RAD 3 Pathway

This pathway has been identified as the excision repair pathway of *S. cerevisiae*. Unrau et al (1971), Resnick and Setlow (1972) and Prakash (1975) have shown that *rad 1* and *rad 2* mutants are unable to excise UV-induced pyrimidine dimers from their DNA. Prakash (1977a,b) has extended this work to show that *rad 3*, *rad 4*, *rad 10* and *rad 16* are also lacking this ability. This biochemical work confirmed the earlier predictions of Kilbey and Smith (1969), based on the similarity of the responses of *rad 1*, *rad 2* and *rad 3* strains of yeast and *hcr*$^-$ (host cell

reactivationless) strains of *E. coli* to both UV-light post-treatments (photoreactivation and dark-holding in non-growth conditions) and to diepoxybutane and nitrosoguanidine. The *her*$^-$ strains had previously been shown to be deficient in the excision of pyrimidine dimers (Howard-Flanders et al, 1966).

Various pleiotropic effects, in addition to their common UV-sensitivity, have been ascribed to mutants in this pathway. Strains carrying *rad 1*, *rad 2*, *rad 3*, *rad 4*, *rad 10* or *rad 22* exhibit increased UV-induced mutation frequencies (Moustacchi, 1969; Resnick, 1969; Averbeck et al, 1974; Cox and Game, 1974; Eckardt et al, 1975; Lawrence and Christensen, 1976). Of these 6, all but *rad 22* also exhibit increased mutation when exposed to nitroquinoline oxide (Prakash, 1976). Homozygous *rad 2* strains show greatly enhanced UV-induced intragenic recombination (Snow, 1968; Hunnable and Cox, 1971; Kowalski and Laskowski, 1975). Strains homozygous for *rad 1*, *rad 3* or *rad 4* also exhibit increases (Snow, 1968; Hunnable and Cox, 1971) but to a lesser extent. Snow (1968) has also reported increased UV-induced homozygosis for *rad 1*, *rad 2*, *rad 3* and *rad 4* homozygotes.

The RAD 18 Pathway

In addition to their common UV- and X-ray sensitivities (Snow, 1967; Cox and Parry, 1968; Resnick, 1969; Lemontt, 1971a), mutants of the genes in this epistasis group (*RAD 18*, *RAD 6*, *REV 1*, *REV 2* (*RAD 5*) and *REV 3*) are characterized by a reduction in UV-mutability (Lemontt, 1971a; Lawrence et al, 1974). Two other genes have been implicated in UV-mutagenesis by this criterion, *RAD 8* and *RAD 9* (Lawrence et al, 1974; Eckardt et al, 1975; Lawrence and Christensen, 1976) and are considered part of the *RAD 18* system.

The data of Lawrence and Christensen (1976) indicate that UV-mutagenesis is essentially dependent on the function of the *RAD 6* and *REV 3* gene products, and that those of the other genes are only required for particular mutational events. The finding of Prakash (1974, 1976) that very low frequencies of reversion mutations are induced in *rad 6* and *rad 9* strains by several chemical mutagens, among them ethylmethane-sulphonate, supports the contention that at least some of the genes in this pathway are involved in induced mutagenesis.

The *RAD 18* system may not however by the only system that resolves chemical-induced lesions mutagenically. Prakash (1974) showed that nitrous acid and nitrosoimidazolidone are mutagenic in *rad 6* and *rad 9* strains.

It should be noted that the extensive induced-mutagenesis studies of Lawrence et al (1974), Lawrence and Christensen (1976) and Prakash (1974, 1976) were all carried out on heterozygous mating-type diploids. The significance of this will be discussed later.

Mutations at some of these loci have also been shown to affect recombination. Homozygous *rad6-1* diploids are blocked in sporulation and do not exhibit X-ray induced mitotic crossing-over (Cox and Game, 1974). Diploid strains homozygous for *rad9-4* show no spontaneous or UV-induced inter- or intragenic recombination (Kowalski and Laskowski, 1975). Lemontt (1971c) has reported that strains homozygous for *rev 1*, *rev 2* or *rev 3* exhibit increased UV-induced homozygosis.

The RAD 51 Pathway

On the basis of interactions following UV-exposure *RAD 50* and *RAD 51* were assigned to this pathway (Cox and Game, 1974). Mutation of either

results in only slight UV-sensitivity suggesting that they play a minor role in the handling of UV-induced damage. In contrast to this is the major involvement of *RAD 50* and *RAD 51* in the processing of ionizing radiation induced damage, as indicated by the sensitivity of *rad 50* and *rad 51* strains to X-rays (see Game and Mortimer, 1974).

X-Ray Sensitivity in Yeast

The assigning of the radiation-sensitive mutants of yeast to repair pathways is complicated by the fact that many of the mutants are sensitive to both UV- and X-irradiation (see Game and Mortimer, 1974). So it is that *rad 6* and *rad 18*, whose epistatic interaction for UV-exposure places them in the same repair sequence, are assigned to different X-ray recovery processes on the basis of their interaction on X-ray exposure (Game and Mortimer, 1974). These authors also showed that the presence of *rad50-1*, *rad51-1*, *rad52-1*, *rad53-1* and *rad54-1* with *rad6-1* and *rad18-1* in the same strain did not render this strain any more sensitive to X-rays than the *rad6-1 rad18-1* double mutant, indicating that no further recovery processes were blocked by any of the additional 5 mutants. The synergistic interaction of *rad 6* or *rad 18* with *rad 51* seen on UV-exposure (Game and Cox, 1973) is not seen here.

The observation of Mortimer (cited in Game and Mortimer, 1974) that a *rad18-2 rad52-1* double mutant is no more sensitive than the septuple mutant or the *rad 6 rad 18* double mutant, mentioned earlier, in conjunction with that of Game (cited in Haynes, 1975) that *rad 52* and *rad 54* are epistatic to *rad 51*, leads to the following scheme for X-ray interactions if one uses the criteria established for UV-interactions: *rad 6*, *rad 51*, *rad 52* and *rad 54* are part of one repair system; *rad 18* is part of another, and *rad 53* is in one of these.

As yet the other 9 X-ray sensitivity genes (*rads 5, 8, 11, 12, 15, 17, 55, 56* and *57*) have not been tested for interactions following X-ray exposure.

Pleiotropic effects of some of the loci concerned primarily with X-ray sensitivity have been reported. Strains carrying *rad 50*, *rad 51*, *rad 52* or *rad 53* are slightly sensitive to UV-light (Game and Mortimer, 1974; Resnick, 1975). In homozygous condition *rads 50-57* result in a reduction of sporulation frequencies and/or spore viability (Game and Mortimer, 1974). Homozygous *rad51-1* strains are deficient in radiation-induced intra- and intergenic recombination (Saeki et al, 1974).

Homozygous *rad 52* strains are deficient in UV- and X-ray-induced intragenic recombination (Resnick, 1975) and are unable to repair DNA double-strand breaks induced by X-irradiation (Ho and Mortimer, 1975; Resnick, 1975). Prakash (1976) has shown that *rad 52* homozygotes exhibit very low frequencies of EMS-induced mutations, implicating this gene, with *rad 6* and *rad 9*, in mutagenic repair. *rad 52* and *rad 50* strains, like *rad 6*, *rad 9* and *rad 18*, also exhibit sensitivity to methylmethanesulphonate (Zimmermann, 1968; Brendel and Haynes, 1973).

The Role of Mating-Type in Repair in Diploids

Mortimer (1958) and Laskowski (1962) showed that diploid yeast heterozygous (*a*/α) at the mating-type locus were more resistant to X-ray inactivation than homozygous (*a*/*a* or α/α) mating-type diploids. Furthermore, the frequency of UV-light induced intragenic recombination in *a*/*a* and α/α diploids is reduced compared to *a*/α diploids (Friis and Roman, 1968). The inference can be drawn from these observations that the presence of both *a* and α alleles in a diploid may produce effects over and above

those expected on the basis of increased ploidy alone. This is seen in the data of Mortimer (1958). The homozygous mating-type diploids, while more sensitive to X-ray inactivation than the *a*/α strains, were nevertheless more resistant than the haploids.

The observation that *a*/α diploids homozygous for *rad 52* (among whose effects are X-ray sensitivity, reduced induced intragenic recombination and reduced sporulation efficiency) do not have increased resistance to X-rays when compared with their *a*/*a* or α/α counterparts (Ho and Mortimer, 1973) and are unable to repair induced-double strand breaks, leads to the conclusion that the "*a*/α effect" is a manifestation of a recombinational process which is *a*/α dependent and requires the function of the *RAD 52* gene product (Ho and Mortimer, 1975; Resnick, 1975). Strains bearing *rad 52* exhibit the mutator phenotype (von Borstel et al, 1968), suggesting a relationship between this repair process and spontaneous mutability.

Mutator Activity and DNA Repair in Yeast

The assumption that DNA lesions, induced in a strain in which a repair pathway is blocked, may be processed via the unblocked routes, is implicit in the scheme used to define the pathways of repair of UV-induced damage (Game and Cox, 1973; Brendel and Haynes, 1973). Such escape will apply primarily when the block is in the first step of a recovery process since incompletely resolved lesions, arriving at a block further down a pathway, may not be a fit substrate for further processing by any system, and result in cell death. If one, or more, of the processes which handle induced lesions do so mutagenically, then a block at the first step of a non-mutagenic route can result in channelling of lesions into the mutagenic repair pathways. This was a major part of the argument of Hastings

et al (1976). Taking it further, they suggested that spontaneous lesions may also be subject to such channelling. They offered this as an explanation of the mutator activity (increased spontaneous mutation rates) observed for some radiation-sensitive mutants of yeast (von Borstel et al, 1968; Suslova and Zakharov, 1971; Brychcy, 1974).

The work of Brychcy lends support to this argument. She showed that *rad 3*, the first step (hypothetically at least) in the excision repair pathway was a mutator, but *rad 1*, *rad 2* and *rad 4*, three later steps, were not.

The first step requirement is not absolute insofar as any block which results in an incompletely processed lesion that is a fit substrate for continued processing by another pathway, may still result in mutation. This provides a not unreasonable rationalization of the mutator activity seen in strains carrying *rad 50*, *rad 51*, *rad 52* or *rad 54* (von Borstel et al, 1968; Suslova and Zakharov, 1971), only one of which (*rad 51*) has been identified as a first step.

Further support for the hypothesis of Hastings et al (1976) comes from their observation that of the eight mutator loci identified all but two also exhibit sensitivity to agents whose effects on DNA are subject to repair. Strains carrying *mut 1* or *MUT 6* are resistant to the 3 agents tested, X-rays, UV-light and methylmethanesulphonate (MMS). Mutants at the remaining 6 loci are all sensitive to MMS, but differ in their responses to insult by X-rays or UV-light: *mut 2* strains are resistant to both X-rays and UV-light; *mut 3* and *mut 4* strains are resistant to X-rays and weakly sensitive to UV; *mut 5* strains show marked sensitivity to X-rays, but only weak sensitivity to UV; *mut 9* strains exhibit slight sensitivity to both, and *mut 10*-bearing strains are sensitive to X-rays but not to UV-light. While the pleiotropic sensitivities of some of

the mutators provide evidence that there is an interaction between the mutator loci and the DNA repair systems of yeast, they do not in themselves provide evidence that the mutators are part of these systems (cf. the *dam* mutants of *E. coli* discussed earlier).

The Problem

When this investigation was initiated, it was clear that if further progress was to be made on the way to understanding the mechanisms of spontaneous mutability in yeast, extensive additional characterization of the mutator mutants would be essential. It seemed clear too that the relationship which appeared to exist between the mutator loci and the DNA repair systems had to be exploited, if for no other reason than that it was then the only reasonable and available context in which to consider them.

The approach taken was to expand the characterization with respect to parameters that would confirm and clarify the relationship of the mutators with the repair systems. The parameters used were UV-mutability, UV-induced recombination and the effect of mating-type constitution on spontaneous mutability. The last was included in the hope that it would indicate whether any channelling relationship exists between the *a*/α-dependent repair process and the mutator loci. The other parameters were used because each of the epistasis groups described earlier is characterized by the particular effect mutation at its constituent loci has on them.

MATERIALS AND METHODS

Strains

The genotypes of the strains used in the construction of stocks for use in this study are contained in Table 1. *a* and α identify the mating-type alleles: *cry 1* identifies strains which are cryptopleurine-resistant; *ura 3, hom 3, his 1, arg 4* and *arg 6, lys 1, ade 2* and *trp 5* identify recessive alleles whose presence in haploids results in nutritional requirement for uracil, homoserine, histidine, arginine, lysine, adenine and tryptophan respectively; *mut* identifies an allele at a mutator locus--the particular locus and allele is identified by the numbers which follow the 3 letter designation.

Each of the *mut*-bearing haploids was crossed to KF164-61. A meiotic product from each of these diploids of the following genotype, α *his1-315 arg 6 lys1-1 trp5-48 mut*, was selected and crossed to a cryptopleurine-resistant isolate of KF164-98. The haploid parents of the diploids used in the characterization studies of *mut*-bearing strains were derived from dissection of this last set of diploids. The genotypes of these haploid strains are contained in Table 2. The diploids used and their haploid parents are listed in Table 3.

TABLE 1

The genotypes of the strains used in preparing stocks for this study

Strain		Genotype	Source
XV357-36D	α	*lys1-1 ade2-1 arg4-17 trp5-48*	R. C. von Borstel
LZ1-9B	a	*ura3 hom3 his1-1 ade1*	E. Savage
T315	α	*his1-315 arg6 trp5-48 ade2-1*	"
XV177-22C	a	*mut1-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	R. C. von Borstel
XV353-6D	a	*mut2-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2 hom3-10*	"
XV195-23A	a	*mut3-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
XV357-36B	a	*mut4-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
XV407-19D	a	*mut5-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
XV374-16B	a	*mut6-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
XV396-14D	a	*mut9-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
XV451-6A	a	*mut10-1 lys1-1 ade2-1 arg4-17 trp5-48 his5-2*	"
KF161-5B	α	*mut4-1 lys1-1 arg4-17 trp5-48*	XV357-36D x XV357-36B (*ade2* revertant)
KF163-6B	a	*mut4-1 ura3 hom3 his1-1 lys1-1 trp5-48*	KF161-5B x LZ1-9B (*arg4-17* revertant)
KF164-61	α	*his1-315 arg6 lys1-1 trp5-48*	KF163-6B x T315
KF164-98	a	*ura3 hom3 his1-1 lys1-1 trp5-48*	KF163-6B x T315

TABLE 2

Genotypes of the haploid strains used to construct the *mut*-bearing diploids for the characterization studies

Strain	Genotype									
KF172- 6B	*mut1-1*	α	+	+	+	*his1-315*	*arg6*	+	*lys1-1*	*trp5-48*
KF172-14D	*mut1-1*	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF172- 1D	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF172-15D	+	α	+	+	+	*his1-315*	*arg6*	+	"	"
KF174- 4A	*mut2-1*	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	*lys1-1*	*trp5-48*
KF174- 4C	*mut2-1*	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF174- 2D	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF174- 7A	+	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	"	"
KF176- 7C	*mut3-1*	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	*lys1-1*	*trp5-48*
KF176- 1B	*mut3-1*	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF176-11C	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF176-11D	+	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	"	"

(cont'd)

TABLE 2 (cont'd)

Strain	Genotype									
KF177- 6D	*mut4-1*	*a*	*cry1*	+	+	*his1-315*	*arg6*	*ade2-1*	*lys1-1*	*trp5-48*
KF177- 5D	*mut4-1*	α	+	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF177- 2A	+	α	+	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF177- 7A	+	*a*	*cry1*	+	+	*his1-315*	*arg6*	*ade2-1*	"	"
KF179- 1A	*mut5-1*	α	+	+	+	*his1-315*	*arg6*	+	*lys1-1*	*trp5-48*
KF179-10C	*mut5-1*	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF179- 1D	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF179- 4A	+	α	+	+	+	*his1-315*	*arg6*	+	"	"
KF181-25C	*mut6-1*	*a*	*cry1*	+	+	*his1-315*	*arg6*	+	*lys1-1*	*trp5-48*
KF181-18D	*mut6-1*	α	+	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF181-11D	+	α	+	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF181-13A	+	*a*	*cry1*	+	+	*his1-315*	*arg6*	+	"	"
KF183-10B	*mut9-1*	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	*lys1-1*	*trp5-48*
KF183- 2B	*mut9-1*	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF183- 2A	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	+	"	"
KF183- 9C	+	α	+	+	+	*his1-315*	*arg6*	*ade2-1*	"	"

(cont'd)

TABLE 2 (cont'd)

Strain									
KF185- 8A	*mut10-1*	α	+	*ura3*	*hom3*	*his1-315*	*arg6*	*ade2-1*	*lys1-1*	*trp5-48*
KF185- 1B	*mut10-1*	*a*	*cry1*	*ura3*	+	*his1-1,315*	+	+	"	"
KF179- 1B	+	α	+	+	*hom3*	*his1-1*	+	*ade2-1*	*lys1-1*	*trp5-48*
KF179- 1C	*mut5-1*	*a*	*cry1*	*ura3*	+	*his1-315*	*arg6*	+	"	"
KF179- 2A	*mut5-1*	α	+	+	*hom3*	*his1-1*	+	+	"	"
KF179- 2B	+	α	+	*ura3*	+	*his1-315*	*arg6*	*ade2-1*	"	"
KF179- 2C	*mut5-1*	*a*	*cry1*	+	+	*his1-315*	*arg6*	+	"	"
KF179- 2D	+	*a*	*cry1*	*ura3*	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF179- 3B	*mut5-1*	α	+	*ura3*	+	*his1-315*	*arg6*	+	"	"
KF179- 3C	+	*a*	*cry1*	+	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF179- 4C	*mut5-1*	α	+	*ura3*	+	*his1-315*	*arg6*	*ade2-1*	"	"
KF179- 4D	*mut5-1*	*a*	*cry1*	+	*hom3*	*his1-1*	+	*ade2-1*	"	"
KF179-11D	+	*a*	*cry1*	+	+	*his1-315*	*arg6*	*ade2-1*	"	"

TABLE 3

The origin of the diploid strains used in the characterization studies

Diploid	Haploid parents
KF186	KF172- 6B x KF172-14D
KF187	KF172- 6B x KF172- 1D
KF188	KF172-15D x KF172-14D
KF189	KF172-15D x KF172- 1D
KF190	KF174- 4A x KF174- 4C
KF191	KF174- 4A x KF174- 2D
KF192	KF174- 7A x KF174- 4C
KF193	KF174- 7A x KF174- 2D
KF194	KF176- 7C x KF176- 1B
KF195	KF176- 7C x KF176-11C
KF196	KF176-11D x KF176- 1B
KF197	KF176-11D x KF176-11C
KF198	KF177- 6D x KF177- 5D
KF199	KF177- 6D x KF177- 2A
KF200	KF177- 7A x KF177- 5D
KF201	KF177- 7A x KF177- 2A

(cont'd)

TABLE 3 (cont'd)

Diploid	Haploid parents
KF202	KF179- 1A x KF179-10C
KF203	KF179- 1A x KF179- 1D
KF204	KF179- 4A x KF179-10C
KF205	KF179- 4A x KF179- 1D
KF217	KF179- 1A x KF179- 1C
KF218	KF179- 2B x KF179-11D
KF219	KF179- 2B x KF179- 2C
KF220	KF179- 2A x KF179- 4D
KF221	KF179- 1B x KF179- 1D
KF222	KF179- 2A x KF179- 2D
KF223	KF179- 3B x KF179- 4D
KF224	KF179- 4C x KF179- 4D
KF225	KF179- 1A x KF179- 4D
KF226	KF179- 3B x KF179-10C
KF227	KF179- 4A x KF179- 3C
KF229	KF179- 1D x KF179- 2B
KF231	KF179- 2D x KF179- 4A
KF206	KF181-25C x KF181-18D
KF207	KF181-25C x KF181-11D
KF208	KF181-13A x KF181-18D
KF209	KF181-13A x KF181-11D
KF210	KF183-10B x KF183- 2B
KF211	KF183-10B x KF183- 2A
KF212	KF183- 9C x KF183- 2B
KF213	KF183- 9C x KF183- 2A
KF214	KF185- 8A x KF185- 1B
KF215	KF185- 8A x KF176-11C
KF216	KF185- 1B x KF176-11D

Media

YD: 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose and 2% Bacto-agar in distilled water.

YG: 1% Bacto-yeast extract, 2% Bacto-peptone, 3% glycerol and 2% Bacto-agar in distilled water.

YA: 1% Bacto-yeast extract, 2% Bacto-peptone and 1% potassium acetate in distilled water.

MC: .67% Bacto-yeast nitrogen base without amino acids, 2% dextrose and 2% Bacto-agar in distilled water, to which is added 20 mg of each of adenine, uracil, arginine, histidine, lysine and methionine, 30 mg of leucine, and 350 mg of threonine in a total of 100.5 ml of stock solutions/litre of medium.

Omission media: MC without one or more of the amino acid or base supplements--in the text referred to as "-(abbreviation for supplement)".

can: -arg medium containing approximately 60 µm/ml canavanine sulphate: 6 ml of filter-sterilized stock solution (1 mg/ml) added to each 1 litre batch of autoclaved medium.

cry: 2 µM cryptopleurine (Chemsea Pty) in YD: 2 ml of filter-sterilized cryptopleurine stock (1 mM) added to 1 litre of autoclaved YD.

Sporulation media:

F^+: 1% potassium acetate, .1% dextrose, .25% yeast extract, 2% Bacto-agar, and amino acids and bases as in MC, in distilled water.

1% KAc: 1% potassium acetate in distilled water.

Mating, Sporulation and Ascus Dissection

For the production of diploids, small amounts of the parental haploid strains were grown for 8-24 hours on YD plates and subsequently mixed together. After 2-4 hours individual zygotes were isolated by micromanipulation using a de Fonbrune micromanipulator and the plate incubated at 26°C for three days. When larger numbers of cells were required the 3 - day zygotic clones were streaked on fresh YD plates.

To obtain asci, actively growing cells were transferred from YD medium to F^+ sporulation medium by streaking or by replica-plating, and incubated at 26°C. F^+ medium allows limited growth of the cells before sporulation. Asci are discernible as early as 24 hours after transfer, but ascus dissection was not usually begun until at least 48 hours.

Asci were prepared for dissection by suspension in 0.5 ml of a 1:10 dilution of glusulase (Endo) in distilled water and incubation at room temperature for 1-2 hours. After this time, the digestion mixture was diluted with 5 ml of sterile distilled water and a small amount of the ascal preparation streaked directly on YD plates. Tetrads were dissected by micromanipulation. After 3 days incubation (5 days if growth was slow) the spore clones were replica-plated to the appropriate media to score phenotypes. Scoring was usually carried out 18-24 hours later.

Plate Test for Detection of the Mutator Phenotype: the "Lassie" Test

Strains to be checked for the mutator phenotype (increased spontaneous mutation) were incubated overnight on YD plates. The cells were then suspended in sterile distilled water, the concentration adjusted to 5×10^6-1×10^7 cells/ml and 0.5 ml plated on each of an MC and a -lys plate. Following incubation for 10 days at 26°C the number of

colonies on each was determined. The difference between the two figures is the number of lysine-independent cells that arose by spontaneous mutation during the lysine-limited growth on MC medium. For the mutator strains involved in this work the difference was ordinarily in excess of 150, and for non-mutators under 50.

Ultraviolet Light (UV) Irradiation

Cells of the strains to be tested were grown on YD at 26°C for 3 days. Cell suspensions were then prepared, their concentration adjusted to 1 x 10^8 cells/ml and a series of dilutions made. For each of the doses used 0.25 or 0.5 ml of a suitably diluted suspension was spread on each of 2 or 4 YD plates to determine survival and intergenic recombination frequencies. The latter was determined by the frequency of red colonies or sectors, which were indicative of homozygosis of *ade2-1*, for which all diploids tested were heterozygous.

The kinetics of UV-induced intragenic recombination was determined by scoring the frequency of histidine prototrophs produced in diploid cells, heteroallelic at *his 1*, by exposure at various doses. Mutation induction was measured by scoring the production of lysine independent cells in these same diploids, all of which were homozygous *lys1-1*. Platings of 0.25 or 0.5 ml of suitably diluted suspensions were made on -his and -lys media.

The ultraviolet light source used was a single low pressure mercury vapour lamp (Sylvania G30T8). The incident energy at the surface of the medium, as determined with a Latarjet Dosimetre, was approximately 1.4 Joules/m^2/sec. The cells were exposed to UV in the dark, 4 plates at a time, and the plates incubated, also in the dark, for 5 days at 26° before scoring.

Gamma Ray Irradiation

The procedures used were essentially the same as those for UV-irradiation. The X-ray source was ^{60}Co in a Gammacell 200 (Atomic Energy of Canada Ltd.). The dose rate was 2 krad/min.

Random Spore Analysis

Diploids were prepared and sporulated as described in "Mating, Sporulation and Dissection". After 4 days incubation on F^+ medium the sporulated mixture was suspended in .5M sodium thioglycolate in .3M Tris (pH 8.8) and incubated at room temperature (about 21°) for 2 hours. The cells were collected by centrifugation, washed once with distilled water, resuspended in 0.2 ml undiluted "Glusulase" (Endo Laboratories), and incubated at 30° for 2 hours. Following dilution in 5 ml distilled water, the cells were pelleted by centrifugation, the supernatant discarded and the pellet resuspended in 5 ml distilled water. The concentration at this point was usually around 10^7 cells/ml.

The separation of spores and the rupture of the remaining diploid cells was accomplished using a continuous flow, French pressure cell (American Instrument Co.). Very effective spore separation was usually obtained when the suspension was expelled from the cell at a pressure of 12000 psi. When greater than 1% unseparated spores and/or unruptured diploids were found when spore counts were made using a hemocytometer, the suspension was passed through the cell again. The pressure cell was sterilized with 1% Roccal (1 hour exposure). Before loading the digested mixture, residual Roccal was diluted by flushing the cell 3-5 times with 40 ml sterile distilled water.

Parameiosis

Single, 3-day zygotic clones of each of the strains to be processed were transferred from YD medium to 5 ml YA (pre-sporulation medium) in 15 x 150 mm tubes and incubated at 26°, with vigorous aeration, for 24 hours. Following dilution in fresh YA medium, to approximately 10^5 cells/ml, 7 ml samples of each were reincubated under the same conditions until the concentration of cells in the cultures reached approximately 10^7 cells/ml. The cells were collected by centrifugation, washed twice with 1% KAc medium, and finally suspended in 7 ml of 1% KAc. Samples of each culture were taken before incubation at 26°, with vigorous aeration, was continued.

Sampling consisted of removing 0.7 ml of suspension; 0.1 ml of this was added to 4% formaldehyde for use in scoring the frequency of asci; 0.1 ml was diluted serially to 10^{-4} and aliquots plated on YD medium to determine relative viability; 0.25 ml was plated on each of 2 -his plates to score prototroph frequency. At 24 and 44 hours diluted samples were plated on -his medium. All plates were scored after 3-4 days incubation at 26°.

Preparation of Homozygous Mating-type Diploids

To assess the effect of mating type on spontaneous mutability in diploids it was necessary to render the mating-type locus homozygous. It was in anticipation of this necessity that cryptopleurine resistance was introduced during stock building. Such resistance can be obtained by mutation at only one locus in *S. cerevisiae*. This gene, designated *CRY 1*, is approximately 2 cM proximal to the mating-type locus on linkage group III (Grant et al., 1974).

The mutant allele used in this study was of spontaneous origin. It was selected by plating 10^8 cells of strain KF164-98 on *cry* medium and incubating the plates at 26° for 5 days. The cryptopleurine resistant (cry R) clones which developed were recloned on YD and retested on *cry* medium. A sample of those which were clearly resistant were crossed to a sensitive strain and the resultant diploids checked for sensitivity to cryptopleurine. A single mutation which was unambiguously recessive was used in stock building.

Homozygous mating type diploids were selected from among *cry R* isolates of *a cry 1/α +* diploids. These are expected to arise primarily as a result of spontaneous mitotic crossing-over proximal to *cry 1*, giving simultaneous homozygosis of *cry 1* and *a* mating type. Cryptopleurine resistant clones which were still heterozygous for mating-type were also isolated. Whether these were the result of recombination or mutation is not known. They did however provide a very useful control in the spontaneous mutation study. To rule out the possibility that the cry R clones were the result of rare sporulation events, these strains were replica-plated to omission media to ensure that none of the other heterozygous recessive markers in the diploids had been uncovered. All such strains were discarded. The mating-type genotypes of all strains used were inferred from mating and sporulation abilities.

RESULTS

The Effects of Mutator Alleles on UV-inactivation, and UV-induction of Mutation and Recombination

mut1-1

The data obtained when the *mut 1*-bearing diploids were tested for UV-inactivation and the induction of mutation and intragenic recombination are contained in Table 4, and plotted in Figures 1, 2 and 3 respectively. The results on induction of homozygosis are presented in Table 5.

For the sake of clarity, only the homozygous *mut 1* diploid's survival curve has been included in Figure 1. It is clear that *mut 1* has no appreciable effect on UV-sensitivity. The mutation induction curves (Figure 2) would seem to indicate that the *mut 1* homozygote exhibits slightly higher UV-mutability at low doses than the wild type or heterozygous strains. A comparison of these results with those that follow for the other sets of diploids will show that the *mut 1/mut 1* curve is well within the range that can be obtained from wild type diploids. The presence of *mut 1* does not appear to affect the frequencies of induced intra- or intergenic recombinants (Figure 3 and Table 5).

It should be noted that the slopes of the final phases of all the UV-induction curves are different from the initial slopes. This was found in all cases where data were obtained for very high doses. The transition between the two took many forms. In some cases if appeared as a simple inflection in the curve. In others a plateau, or a decline, intervened. The possible significance of these will be discussed later.

TABLE 4

The Effect of *mut1-1* on UV inactivation and Prototroph Induction[1]

A. KF186 (*mut1-1/mut1-1 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(789)[4]	.091	(143)[4]	.196	(1545)[4]
21	102	(801)	2.96	(244)	2.91	(241)
42	87.7	(692)	6.82	(478)	4.66	(336)
63	52.0	(410)	9.99	(411)	6.19	(262)
84	20.0	(316)	9.66	(154)	5.88	(96)
105	5.3	(83)	13	(55)	8.0	(34)
126	1.0	(79)	32	(50)	6.1	(10)
147	.274	(216)	13	(27)	2	(1)
168	.027	(43)	61	(13)	42	(9)
189	.0024	(19)	160	(6)	630	(12)
210	.0011	(17)	140	(12)	240	(2)

[1] Strains constructed from meiotic products of KF172

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 4 (cont'd)

B. KF187 (*mut1-1/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(813)	.12	(195)	.11	(93)
21	96.9	(788)	.92	(82)	1.21	(104)
42	94.6	(769)	2.84	(227)	2.69	(215)
63	63.2	(514)	4.39	(232)	4.09	(216)
84	30.0	(488)	4.96	(124)	4.03	(101)
105	10.6	(172)	6.4	(56)	2.8	(25)
126	1.55	(126)	15	(37)	9.8	(25)
147	.282	(229)	15	(35)	13	(6)
168	.045	(73)	30	(11)	100	(38)
189	.0043	(35)		(0)	140	(5)
210	.0019	(31)	320	(49)	190	(3)

C. KF188 (*mut1-1/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1068)	.046	(98)	.017	(37)
21	96.2	(1027)	2.40	(252)	1.31	(137)
42	90.0	(961)	6.37	(617)	3.18	(308)
63	51.9	(554)	10.9	(603)	5.67	(315)
84	20.1	(429)	13.0	(279)	8.74	(188)
105	6.37	(136)	19.6	(133)	4.8	(33)
126	1.12	(120)	16	(39)	8.7	(21)
147	.175	(187)	78.6	(147)	20	(7)
168	.014	(29)	260	(37)	260	(37)
189	.0025	(27)	280	(15)	410	(11)
210	.0016	(34)	1050	(178)	200	(4)

TABLE 4 (cont'd)

D. KF189 (+ / + ; *his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(833)	.019	(31)	.005	(4)
21	97.5	(812)	.93	(77)	1.27	(103)
42	102	(846)	2.31	(197)	2.61	(221)
63	63.3	(527)	4.82	(255)	4.19	(221)
84	29.6	(493)	6.07	(150)	3.7	(92)
105	8.64	(144)	8.9	(64)	4.4	(32)
126	2.10	(175)	14	(48)	7.4	(26)
147	.389	(324)	25	(82)	6	(4)
168	.0900	(150)	13	(10)	29	(22)
189	.0187	(156)	32	(10)	64	(10)
210	.0030	(50)	280	(70)	160	(4)

TABLE 5

The Effect of *mut1-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)					
		21 J/m^2		42 J/m^2		63 J/m^2	
KF186	*mut1-1/mut1-1*	2.2 (18)[2]	102[3]	3.6 (25)	87.7	9.0 (37)	52.0
KF187	*mut1-1/ +*	2.0 (16)	96.9	2.9 (22)	94.6	7.8 (40)	63.2
KF188	*+ /mut1-1*	1.6 (16)	96.2	5.6 (54)	90.0	7.4 (41)	51.9
KF189	*+ / +*	1.7 (14)	97.5	3.7 (31)	102	7.0 (37)	63.3

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

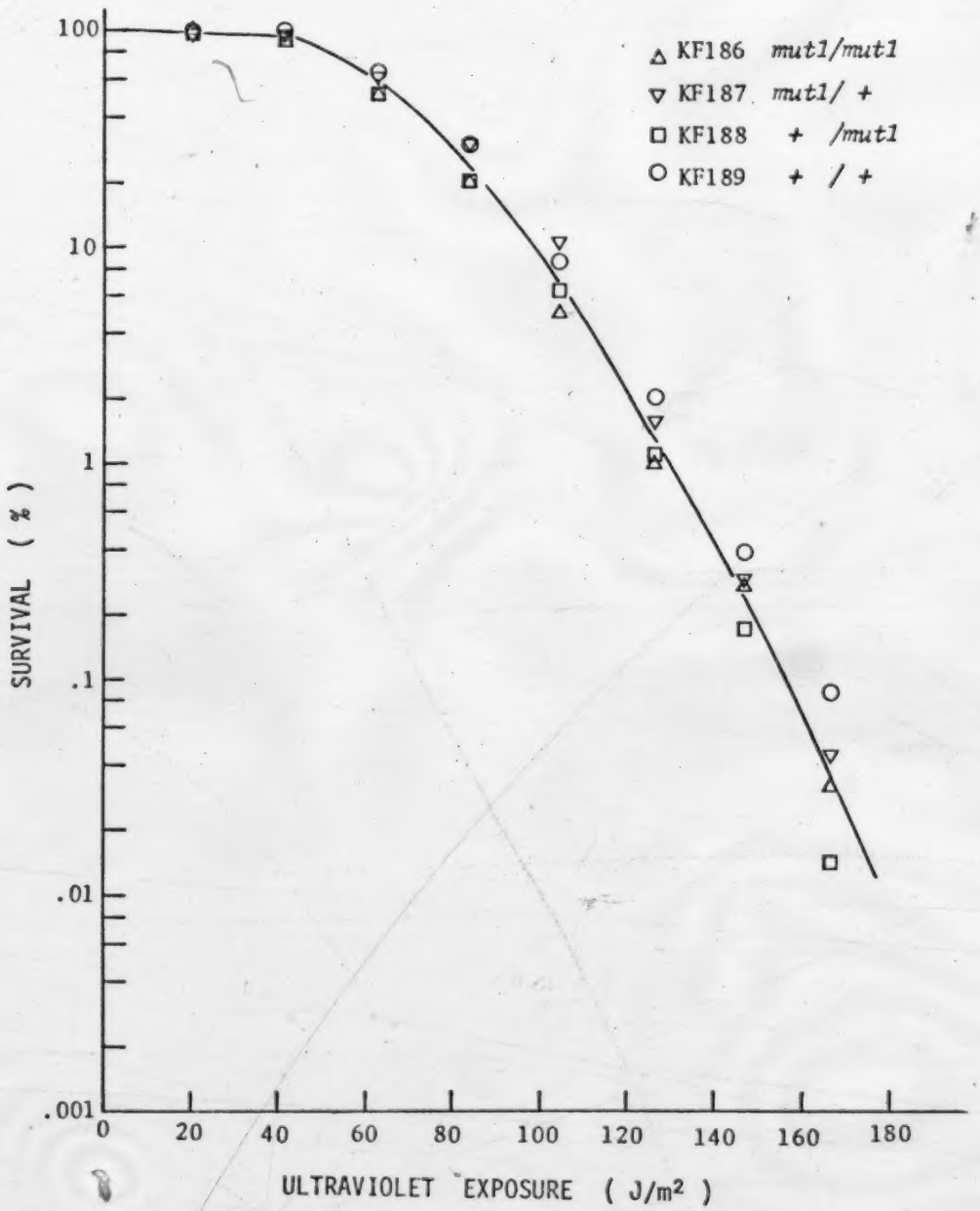

Figure 1 Survival after UV-irradiation of *mut1-1* bearing diploid strains

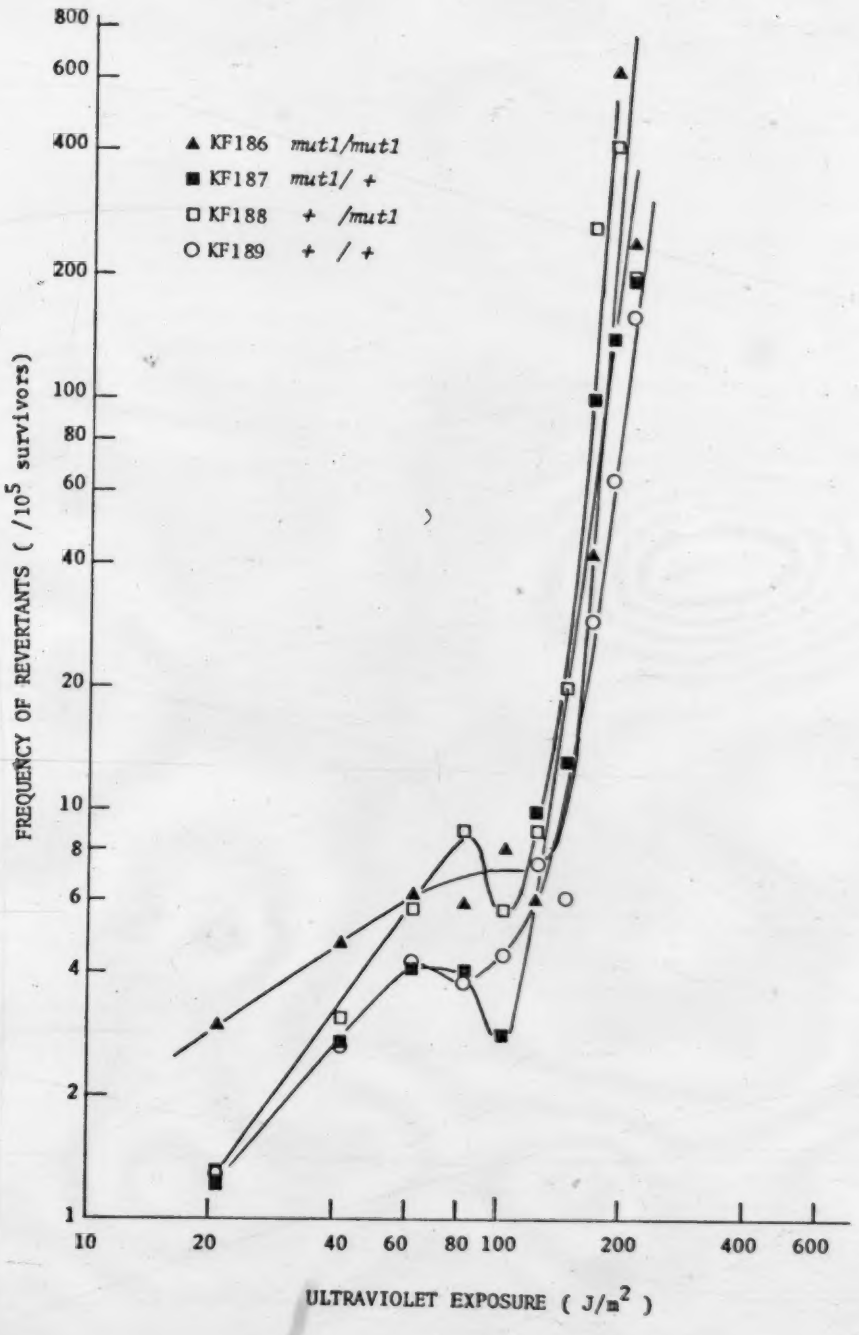

Figure 2 *lys1-1* reversion dose-response curves for *mut1-1*-bearing diploid strains

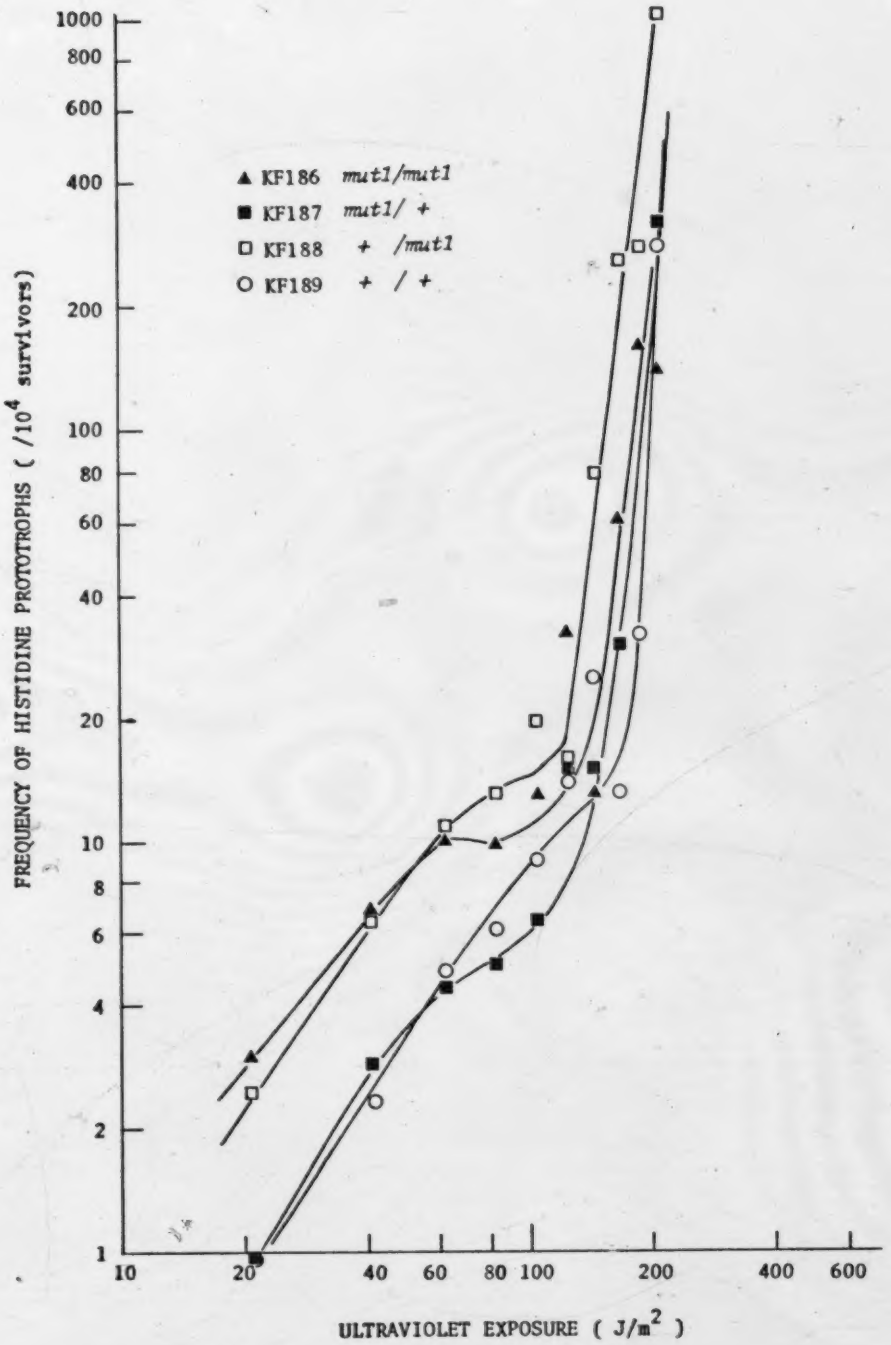

Figure 3 Dose-response curves for intragenic recombination in heteroallelic *his 1* diploid strains carrying *mut1-1*

mut2-1

The results collected for KF190, a *mut2-1* homozygote, and 2 related strains are presented in Tables 6 and 7 and Figures 4-6. A very slight UV-sensitivity is seen for the *mut 2* homozygote (Figure 4). The frequencies of lysine revertants for the *mut 2* homozygote are plotted in Figure 5. Little difference is seen between this curve and those of the heterozygotes and the wild type from the *mut 1* set. Intragenic recombination induction for the *mut 2* strains do not differ appreciably over the range 21-84 J/m^2 (Figure 6). Their responses at doses in excess of this are quite variable. Intergenic recombination frequencies (Table 7) are also unaffected by *mut 2*.

mut3-1

Tables 8 and 9 and Figures 7-9 contain the data collected on the *mut 3* strains. The weak sensitivity reported for *mut 3* strains (Hastings et al, 1976) is not discernible in Figure 7. Apart from the variability in the transition phase, both the mutation and the recombination induction curves (Figures 8 and 9 respectively) indicate no effect of *mut 3*. No obvious influence of *mut 3* on induced homozygosis is seen in Table 9.

mut4-1

Homozygous *mut 4* diploids exhibit an increased sensitivity to UV-light (Table 10 and Figure 10). The inflection in the *mut 4/mut 4* curve at 63 J/m^2 indicates the presence of a resistant sub-population of cells. Mutation induction is essentially unaffected by the presence of *mut 4* (Table 10 and Figure 11).

The wide range of frequencies of induced histidine prototrophs, and the slight elevation of the *mut 4/mut 4* curves (Table 10 and Figure 12) prompted a second look. The additional data, contained in Table 11 failed

TABLE 6

The Effect of *mut2-1* on UV inactivation and Prototroph Induction[1]

A. KF190 (*mut2-1/mut2-1 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(642)[4]	.08	(105)[4]	.61	(405)[4]
21	95.0	(610)	1.69	(108)	1.58	(135)
42	91.6	(588)	4.17	(250)	3.67	(253)
63	70.7	(454)	5.43	(250)	5.98	(300)
84	39.3	(505)	6.93	(177)	6.89	(190)
105	14.8	(190)	4.7	(45)	4.5	(49)
126	4.58	(294)	3.5	(21)	7.1	(45)
147	.510	(655)	4.7	(31)	7.0	(10)
168	.179	(230)	8.7	(10)	48	(56)
189	.0364	(234)	13	(6)	67	(16)
210	.00283	(182)	30	(27)	44	(4)

[1] Strains constructed from meiotic products of KF174

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 6 (cont'd)

B. KF190 (*mut2-1/mut2-1 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(945)	.04	(69)	
21	93.3	(882)	1.86	(1675)	
42	92.2	(871)	4.87	(855)	
63	65.9	(623)	8.27	(1036)	Not scored
84	17.8	(1683)	12.7	(2144)	
105	6.95	(657)	6.43	(425)	
126	1.17	(2219)	13.7	(304)	

C. KF192 (*mut2-1/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(1016)	.09	(179)	
21	94.2	(957)	2.04	(2258)	
42	96.2	(977)	5.68	(1128)	Not scored
63	80.2	(815)	9.20	(1514)	
84	33.6	(3410)	11.9	(4052)	
105	13.7	(1389)	17.7	(2465)	

TABLE 6 (cont'd)

D. KF193 (+ / + ; *his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(588)	.03	(38)	
21	104	(614)	1.70	(102)	
42	105	(618)	4.56	(270)	
84	71.8	(844)	6.68	(283)	Not scored
105	38.8	(456)	6.42	(147)	
126	10.5	(617)	25.0	(309)	
147	2.39	(2808)	31.0	(435)	
168	1.00	(1180)	19.7	(116)	
189	.124	(729)	39	(57)	
210	.0315	(1854)	238	(442)	

TABLE 7

The Effect of *mut2-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)					
		21 J/m^2		42 J/m^2		63 J/m^2	
KF190	*mut2-1/mut2-1*	2.3 (11)[2]	93.3[3]	7.0 (29)	92.2	8.6 (27)	65.9
KF190	*mut2-1/mut2-1*	1.6 (11)	95.0	3.5 (22)	91.6	8.6 (40)	70.7
KF191	*mut2-1/* +	1.3 (6)	94.2	4.3 (19)	96.2	5.2 (20)	80.2
KF193	+ / +	1.3 (13)	91.3	2.7 (18)	94.9	4.5 (23)	80.0
KF193	+ / +	1.1 (7)	104	1.8 (11)	105	8.3 (70)	71.8

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

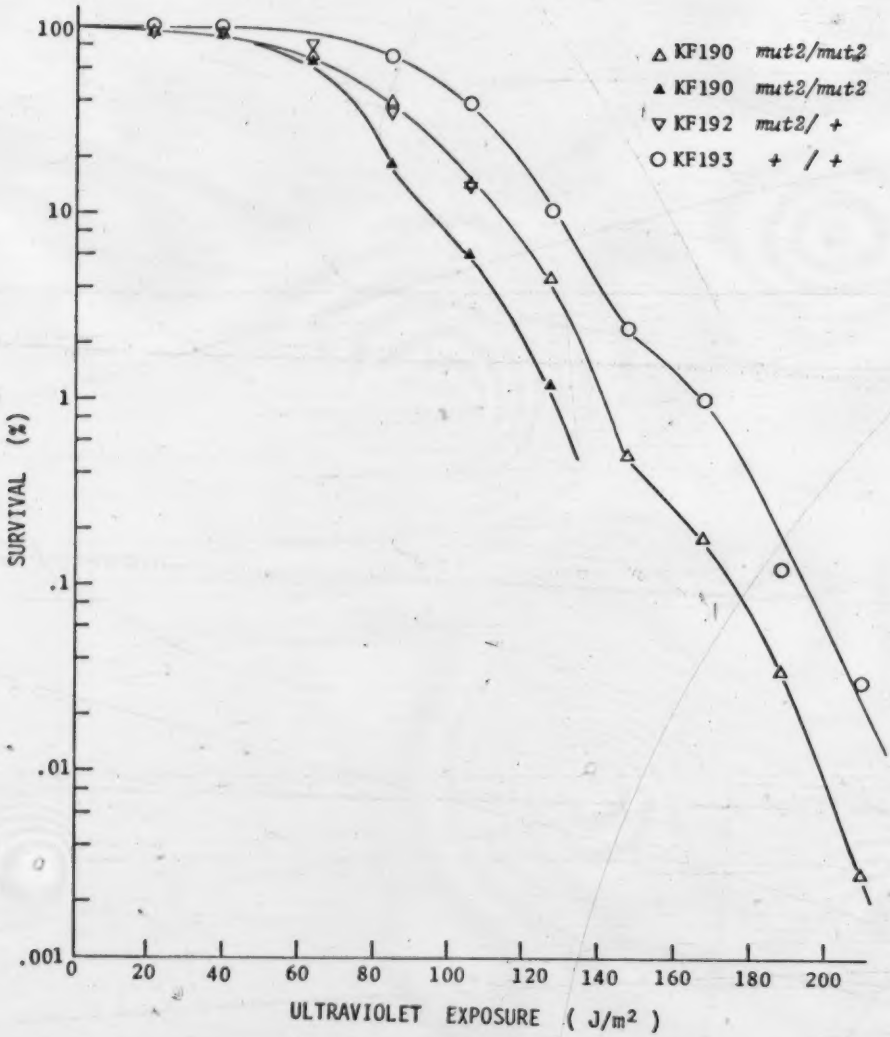

Figure 4 Survival after UV-irradiation of *mut2-1* bearing diploid strains

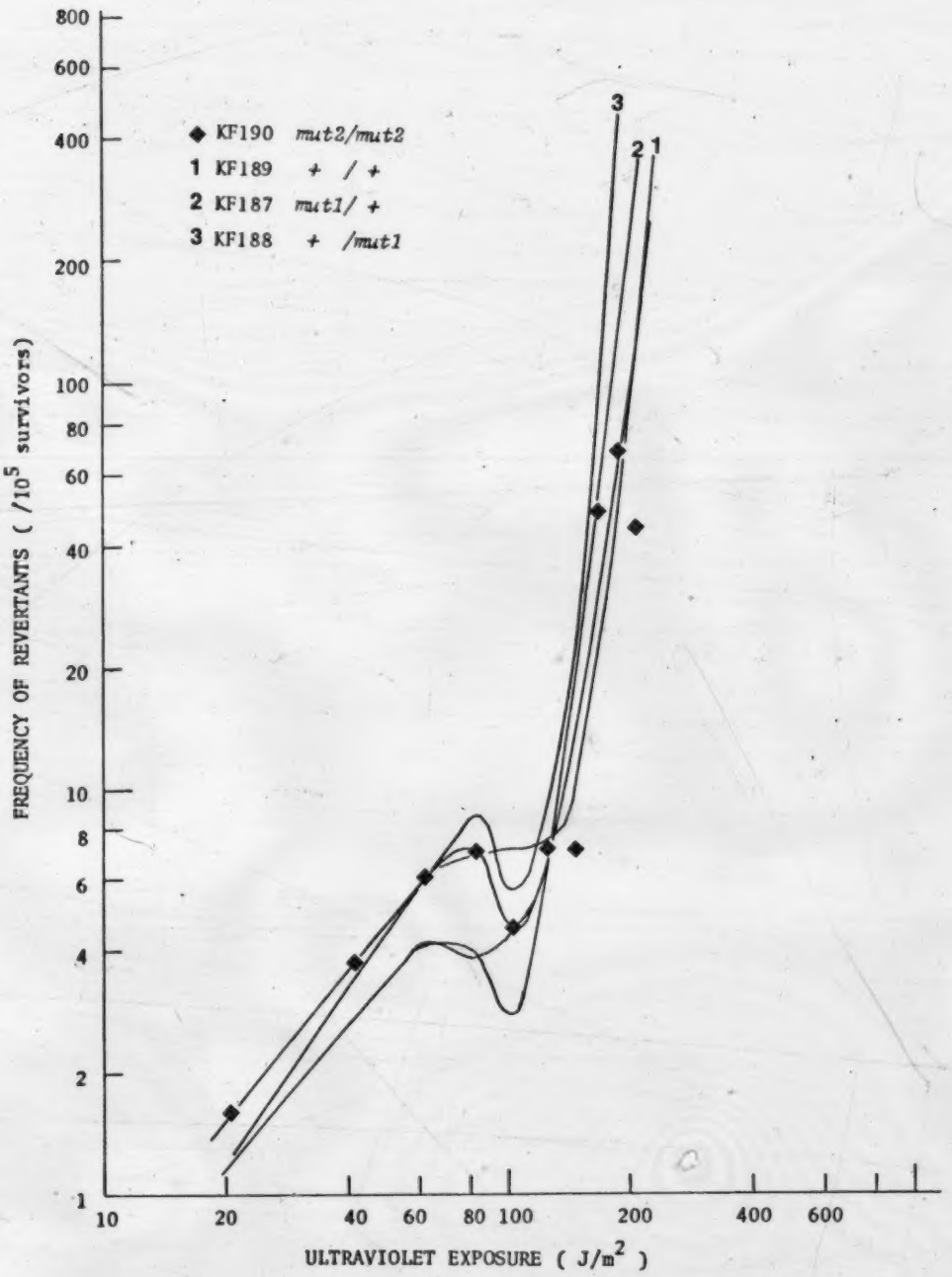

Figure 5 *lys1-1* reversion dose-response curves for a *mut2-1* homozygous diploid strain. Curves 1, 2 and 3 are the wild type and heterozygote curves from the *mut 1* set.

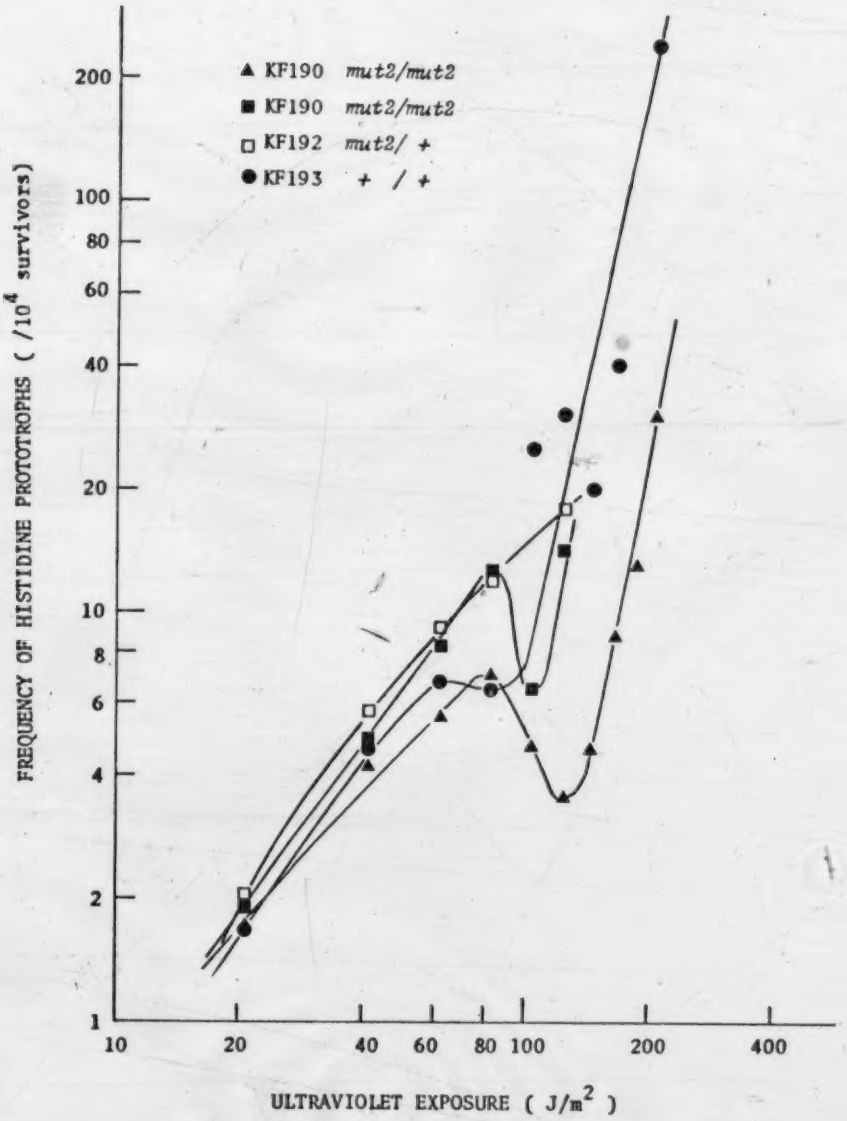

Figure 6 Dose-response curves for intragenic recombination in heteroallelic *his 1* diploid strains carrying *mut2-1*

TABLE 8

The Effect of *mut3-1* on UV inactivation and Prototroph Induction[1]

A. KF194 (*mut3/mut3 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(486)[4]	.15	(74)[4]	.91	(44)[4]
21	91.4	(444)	1.15	(575)	2.04	(131)
42	84.6	(411)	4.25	(1809)	5.88	(279)
63	47.1	(229)	7.05	(1649)	9.09	(230)
84	21.6	(105)	12.9	(1369)	13.2	(148)
126	3.5	(17)	8.38	(145)	8.50	(16)
168	.074	(36)			79.7	(29)
210	.002	(1)	200	(20)	300	(3)

[1] Strains constructed from meiotic products of KF176

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 8 (cont'd)

B. KF194 (*mut3/mut3 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(483)	.06	(29)	.31	(15)
21	86.7	(419)	1.19	(525)	1.7	(84)
42	87.0	(420)	3.30	(1410)	4.67	(209)
63	53.6	(259)	6.55	(1711)	7.10	(192)
84	27.3	(132)	8.77	(1166)	6.6	(91)
126	3.7	(18)	7.90	(153)	4.1	(8)
168	.0269	(131)	34.1	(447)	18	(24)
210	.0025	(12)	25.8	(31)	70	(8)

C. KF195 (*mut3/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(405)	1.77	(718)	.37	(15)
21	99.2	(402)	.26	(816)	1.25	(65)
42	106	(428)	3.27	(2158)	4.85	(223)
63	65.7	(266)	6.67	(2246)	9.25	(256)
84	29.9	(121)	10.3	(1470)	13.6	(169)
126	2.2	(9)	17.9	(163)	20	(18)
168	.09	(38)			60	(23)
210	.01	(5)	140	(70)	80	(4)

TABLE 8 (cont'd)

D. KF196 (*mut3/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(382)	.15	(59)	.08	(3)
21	99.7	(381)	1.51	(638)	1.4	(58)
42	89.3	(341)	5.04	(1771)	5.73	(198)
63	74.9	(286)	7.91	(2305)	8.70	(251)
84	40.3	(154)	11.7	(1826)	13.3	(206)
126	3.9	(15)	17.9	(271)	11	(17)
168	.15	(57)	79.8	(456)	32	(18)
210	.008	(3)	60	(18)	67	(2)

E. KF197 (*+ / + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(358)	.23	(80)	.08	(3)
21	98.9	(354)	4.34	(1615)	1.6	(58)
42	96.9	(347)	8.88	(3159)	3.72	(132)
63	41.9	(150)	17.3	(2620)	6.1	(93)
84	22.3	(80)	17.1	(1382)	7.3	(59)
126	2.8	(10)	15.4	(154)		(0)
168	.084	(30)	75.8	(228)	23	(7)
210	.008	(3)	100	(30)		(0)

TABLE 9

The Effect of *mut3-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)					
		21 J/m^2			42 J/m^2		63 J/m^2		
KF194	*mut3-1/mut3-1*	.7	(4)[2]	91.4[3]	3.6	(15)	84.6	8.7	(20)	47.1
KF194	*mut3-1/mut3-1*	2.6	(11)	86.7	4.0	(17)	87.0	5.0	(13)	53.6
KF195	*mut3-1/* +	.5	(2)	99.2	4.0	(17)	106	5.6	(15)	65.7
KF196	+ */mut3-1*	1.8	(7)	99.7	3.5	(12)	89.3	5.9	(17)	74.9
KF197	+ / +	1.4	(5)	98.9	4.0	(14)	96.9	9.3	(14)	41.9

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

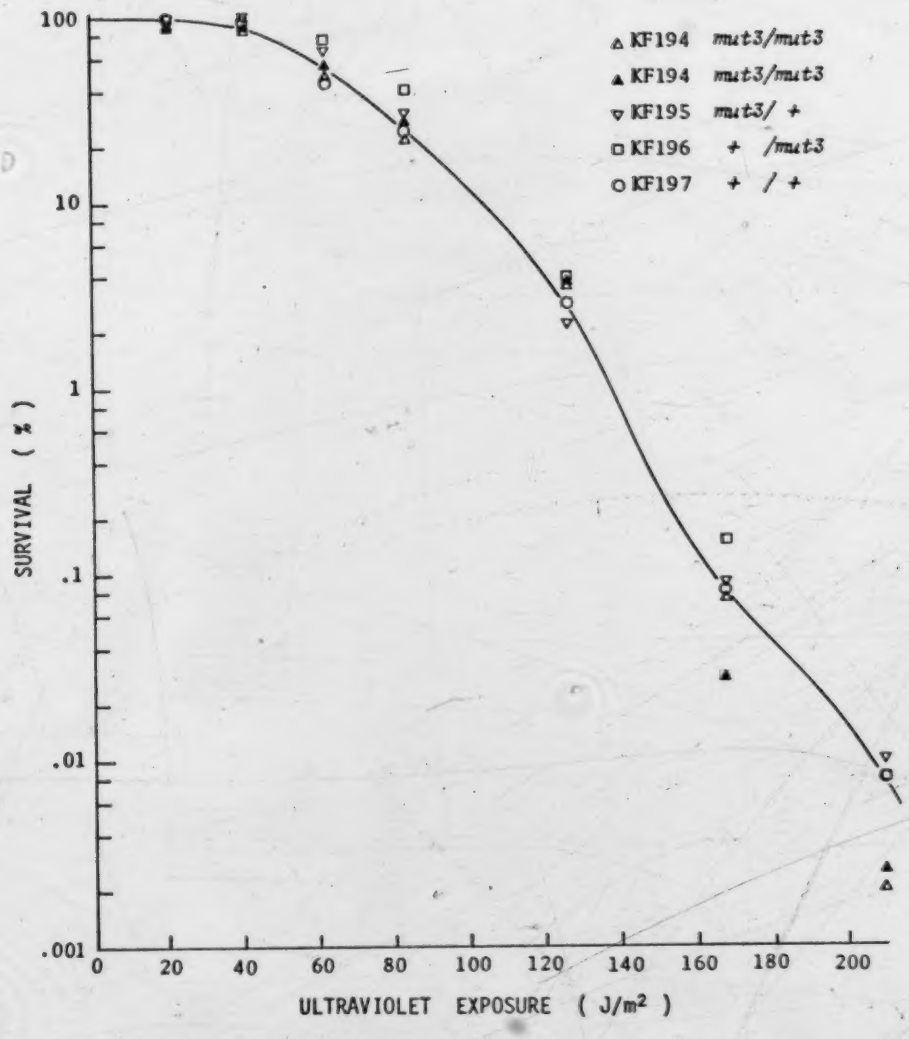

Figure 7 Survival after UV-irradiation of *mut3-1* bearing diploid strains
Only the wild type curve is included.

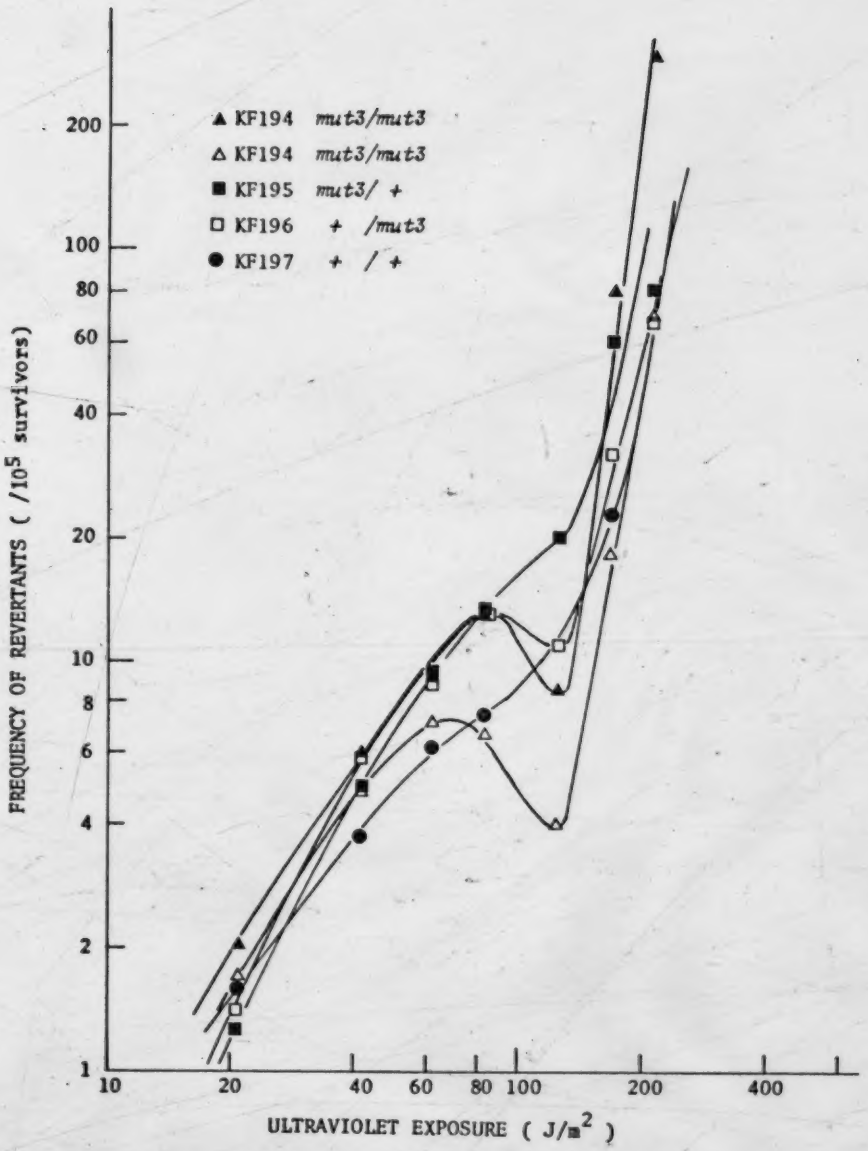

Figure 8 *lys1-1* reversion dose-response curves for *mut3-1*-bearing diploid strains

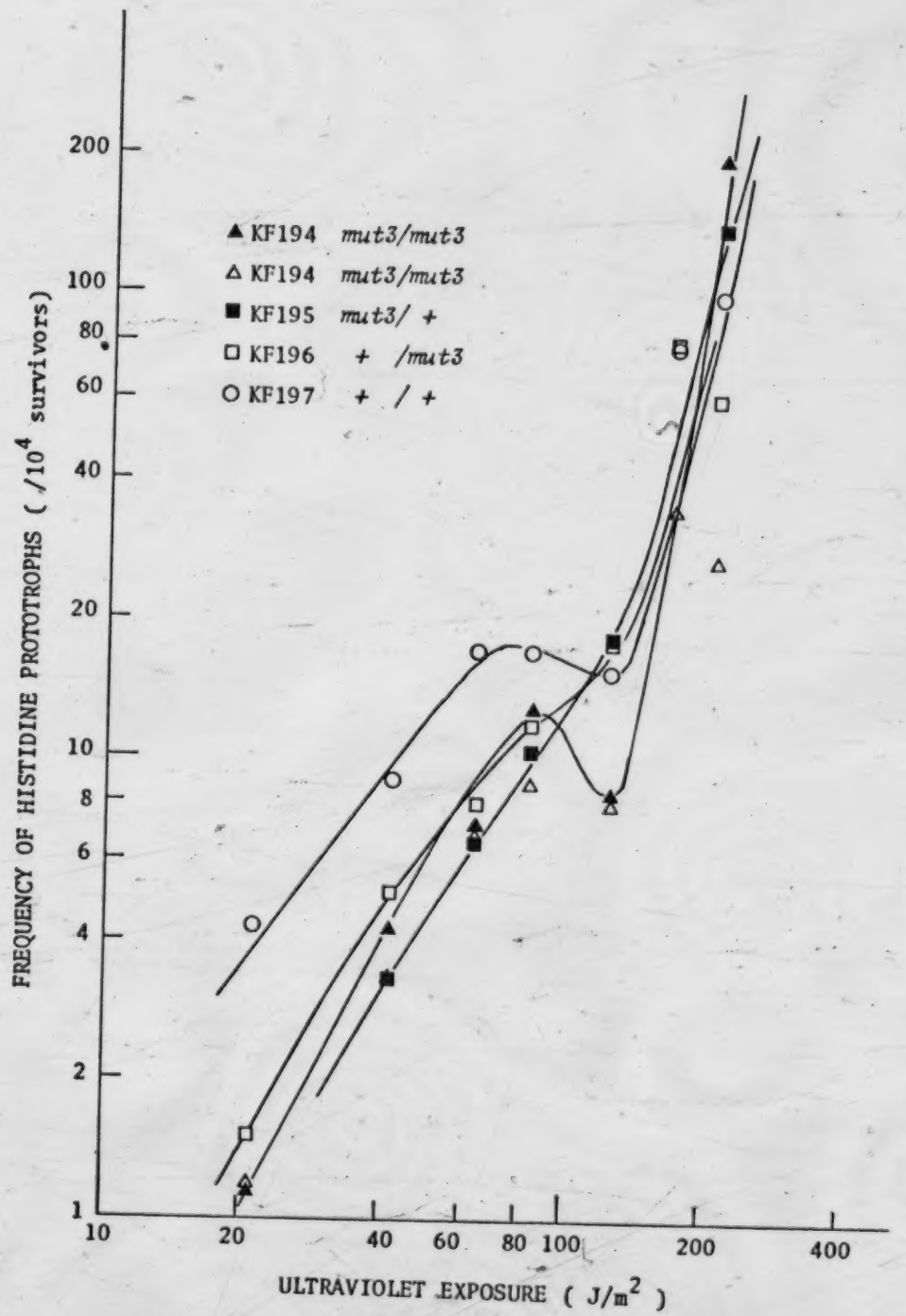

Figure 9 Dose-response curves for intragenic recombination in heteroallelic *his 1* diploid strains carrying *mut3-1*

TABLE 10

The Effect of *mut4-1* on UV inactivation and Prototroph Induction[1]

A. KF198 (*mut4/mut4 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(530)[4]	.013	(7)[4]	.094	(5)[4]
21	89.4	(474)	6.26	(297)	.77	(41)
42	60.9	(323)	14.7	(475)	2.4	(81)
63	17.4	(92)	44.1	(406)	8.3	(77)
84	7.7	(41)	51.7	(212)	8.4	(35)
126	.76	(4)	30	(12)	30	(12)

[1] Strains constructed from meiotic products of KF177

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 10 (cont'd)

B. KF198 (*mut4/mut4 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(561)	.12	(66)	.02	(1)
21	83.1	(466)	4.72	(225)	1.4	(64)
42	59.2	(332)	12.0	(401)	3.96	(132)
63	17.8	(100)	22.9	(230)	9.3	(93)
84	7.3	(41)	16	(66)	4.6	(19)
126		(0)		(5)		(2)

C. KF199 (*mut4/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(576)	.076	(44)	
21	92.5	(533)	3.35	(183)	
42	85.2	(491)	8.05	(399)	
63	44.6	(257)	11.4	(296)	Not scored
84	18.8	(108)	9.37	(102)	
126	.5	(3)	20	(6)	

TABLE 10 (cont'd)

D. KF200 (*mut4/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(565)	.032	(18)		(0)
21	105	(593)	1.3	(76)	.79	(47)
42	99.3	(561)	3.71	(210)	2.41	(135)
63	70.4	(398)	6.53	(261)	5.15	(205)
84	39.5	(223)	7.28	(163)	5.92	(132)
126	6.4	(36)	3.3	(12)	5.83	(21)
168	.18	(1)	10	(1)		(0)

E. KF201 (*+ / + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(917)	.016	(15)	.01	(1)
21	100	(917)	1.60	(149)	.76	(71)
42	83.8	(768)	3.37	(260)	2.11	(163)
63	60.5	(555)	6.68	(372)	3.05	(170)
84	31.4	(288)	7.55	(218)	3.98	(115)
126	3.1	(28)	8.19	(23)	2.85	(8)
168	.1	(1)	30	(3)		(0)

TABLE 11

The Effect of *mut4-1* on UV-inactivation and UV-induced Intragenic Recombination[1]

A. KF198 (*mut4-1/mut4-1 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[2]	
0	100	(527)[3]	.075	(79)[3]
21	97.2	(512)	3.23	(1683)
42	67.6	(356)	10.1	(1820)
63	22.8	(240)	18.7	(1125)
84	2.6	(27)	58.4	(395)

B. KF201 (*+ / + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)	
0	100	(197)	.11	(44)
21	100	(199)	4.76	(1940)
42	78.2	(154)	11.6	(1795)
63	47.0	(185)	15.2	(1416)
84	10.4	(41)	43.7	(898)

C. KF199 (*mut4-1/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)	
0	100	(218)	.060	(26)
21	95.9	(209)	2.75	(588)
42	94.0	(205)	6.09	(1260)
63	62.8	(273)	12.1	(1662)
84	30.7	(134)	28.6	(1920)

[1] Strains constructed from meiotic products of KF177

[2] Induction frequencies corrected for spontaneous level

[3] Colony counts on which frequencies based

TABLE 12

The Effect of *mut4-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)					
		21 J/m^2			42 J/m^2		
KF198	*mut4-1/mut4-1*	3.6	(17)[2]	89.4[3]	6.2	(20)	60.9
KF198	*mut4-1/mut4-1*	2.1	(10)	83.1	4.2	(14)	59.2
KF200	+ */mut4-1*	1.4	(8)	105	4.1	(23)	99.3
KF201	+ / +	1.6	(15)	100	4.4	(34)	83.8

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)		
		63 J/m^2		
KF198	*mut4-1/mut4-1*	5.4	(5)	17.4
KF198	*mut4-1/mut4-1*	2.0	(2)	17.8
KF200	+ */mut4-1*	3.3	(13)	70.4
KF201	+ / +	5.6	(31)	60.5

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

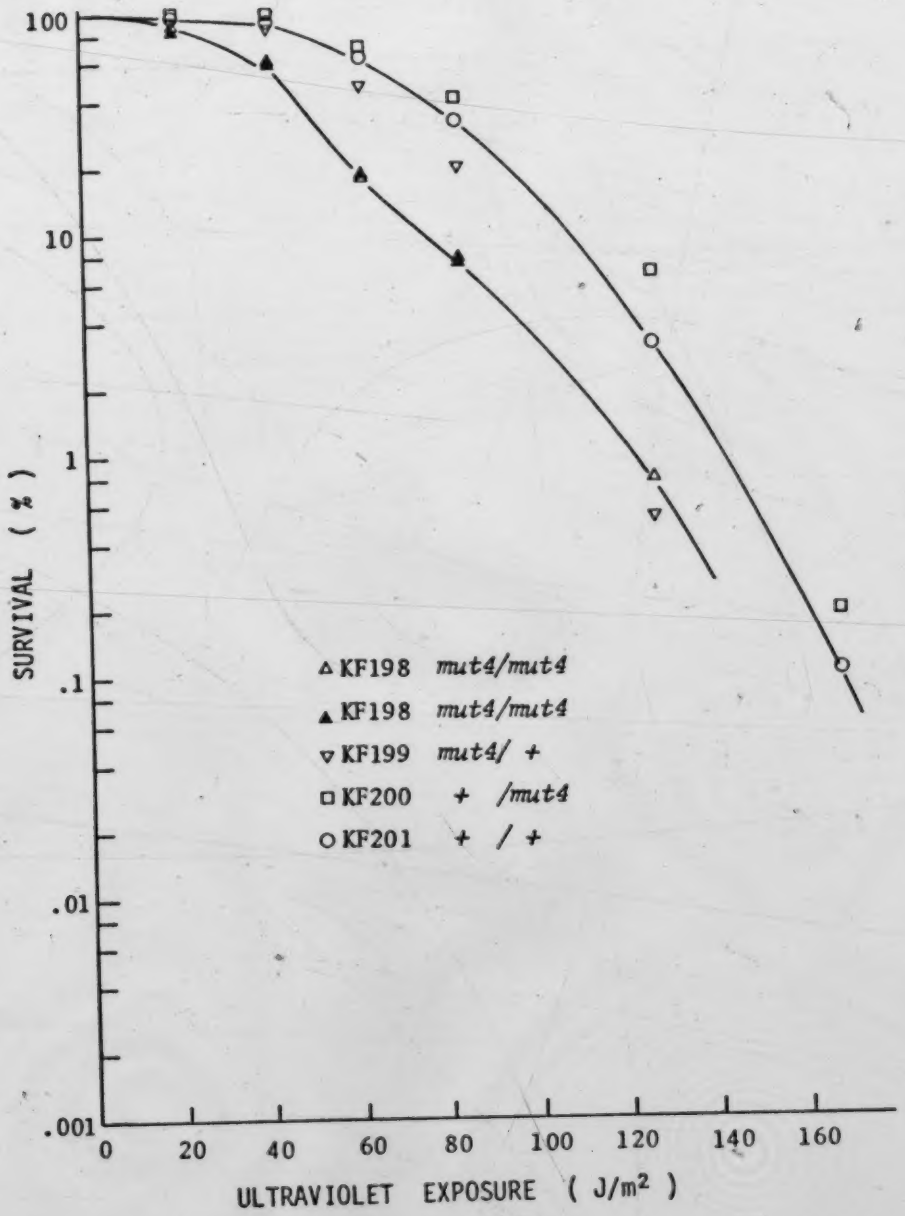

Figure 10 Survival after UV-irradiation of *mut4-1* bearing diploid strains

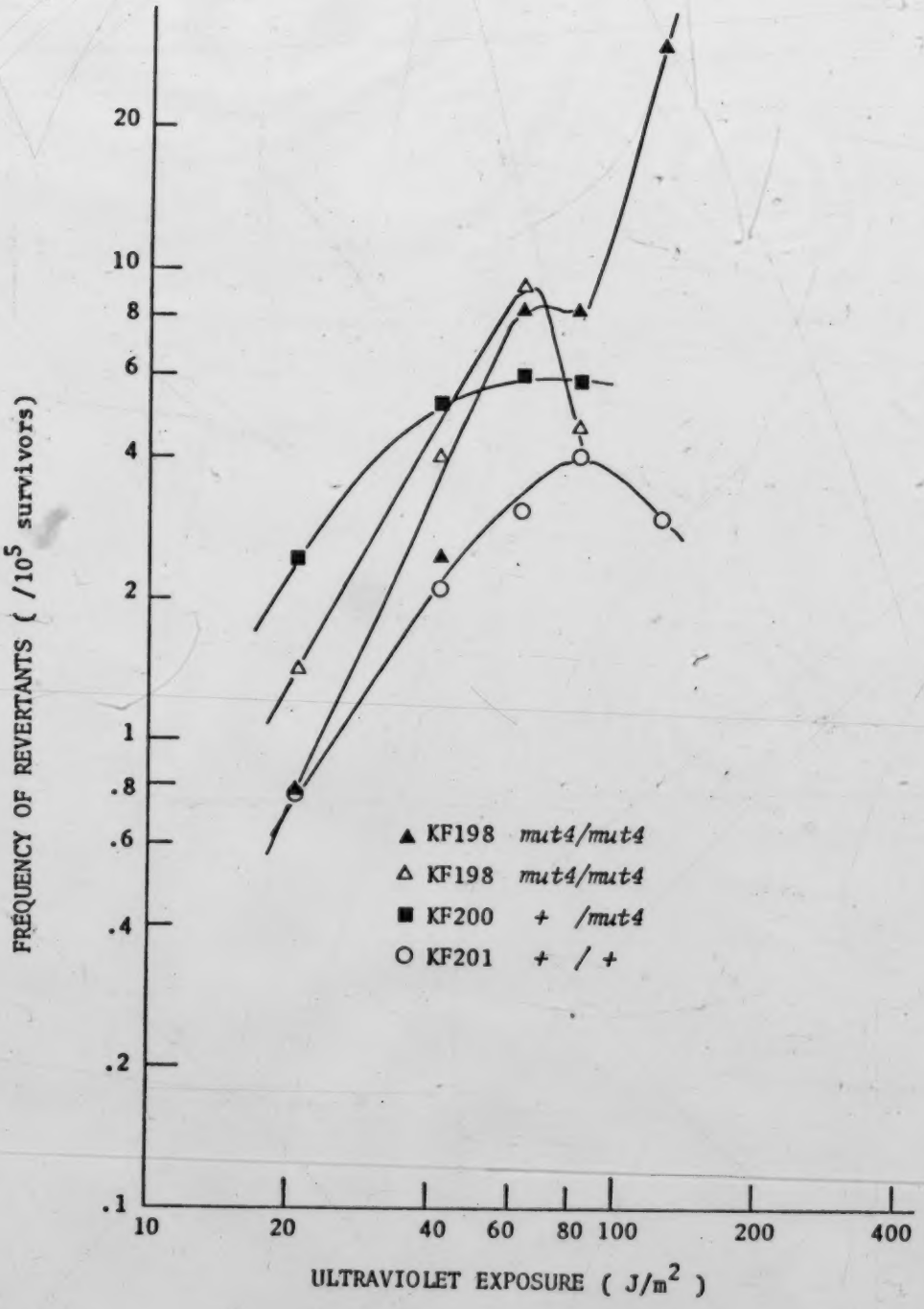

Figure 11 *lys1-1* reversion dose-response curves for *mut4-1*-bearing diploid strains

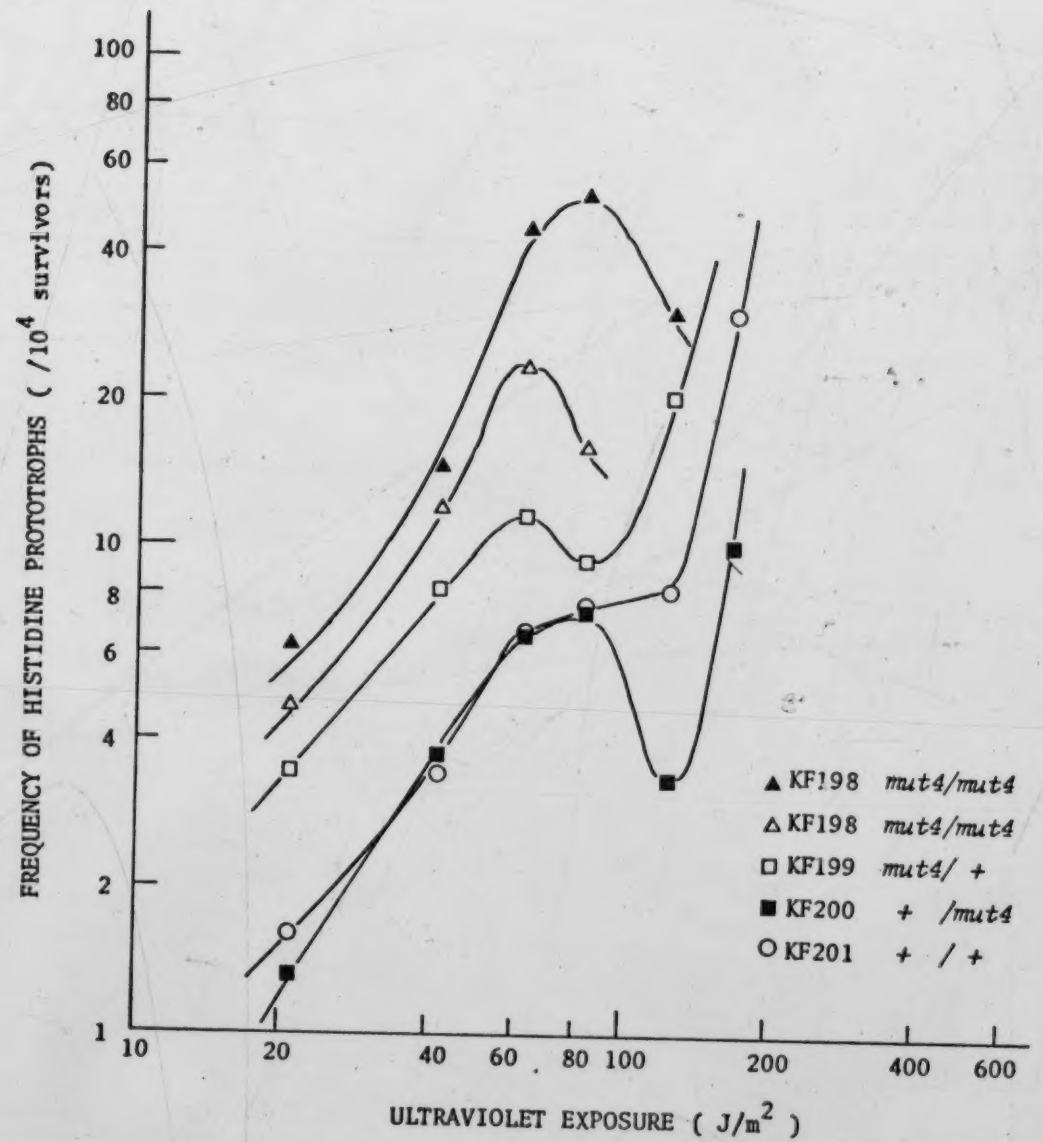

Figure 12 Dose response curves for intragenic recombination in hetero-allelic *his 1* diploid strains carrying *mut4-1*

to show the same result. No major effect of *mut 4* on UV-induced homozygosis is apparent (Table 12).

mut6-1

The results obtained from the testing of the strains carrying *mut 6* are contained in Tables 13 and 14, and Figures 13-15. Homozygous *mut 6* diploids do not exhibit any marked sensitivity to UV-light (Figure 13). The mutation and recombination induction curves (Figures 14 and 15 respectively) show no effect of *mut 6* at low doses. The variability in the transition region of the curve is again pronounced. The presence of *mut 6* has no consistent effect on induced intergenic recombination (Table 14).

mut9-1

Homozygous *mut9-1* strains exhibit slight, and somewhat variable, sensitivity to UV-inactivation (Table 15 and Figure 16). No effect of *mut 9* on UV-induced mutation (Table 15 and Figure 17) or UV-induced homozygosis (Table 16) was observed. The induced intragenic recombination frequencies are lower for *mut 9* homozygotes than the heterozygotes or wild type (Table 15 and Figure 18). Comparison of these curves with those in Figure 24, which were obtained from wild type diploids from *mut 5* background, indicates that the *mut 9/mut 9* frequencies are nevertheless within the range that can be obtained from wild types.

TABLE 13

The Effect of *mut6-1* on UV inactivation and Prototroph Induction[1]

A. KF206 (*mut6/mut6 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(696)[4]	.059	(41)[4]	.17	(12)[4]
21	101	(700)	2.73	(195)	.72	(62)
42	99.3	(691)	6.82	(477)	2.06	(154)
63	71.5	(497)	12.9	(648)	3.87	(201)
84	42.9	(298)	16.9	(508)	5.80	(178)
126	9.10	(63)	15	(94)	3.32	(22)
168	1.4	(10)	3.0	(3)		(0)

[1] Strains constructed from meiotic products of KF181

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 13 (cont'd)

B. KF206 (*mut6/mut6 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(571)	.075	(43)	.18	(10)
21	99.5	(568)	2.84	(166)	1.07	(71)
42	96.3	(550)	7.32	(407)	1.89	(114)
63	77.8	(444)	12.1	(540)	3.58	(167)
84	38.5	(220)	18.8	(415)	5.23	(120)
126	8.4	(48)	13	(63)	5.7	(28)
168	1.6	(9)	2.2	(2)	3.1	(3)

C. KF207 (*mut6/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(462)	.14	(63)	.15	(7)
21	106	(488)	2.26	(117)	1.0	(58)
42	96.3	(445)	6.29	(286)	3.11	(145)
63	97.2	(449)	8.37	(382)	4.48	(208)
84	46.5	(215)	13.1	(283)	6.68	(147)
126	9.1	(42)	13	(55)	3.4	(15)
168	1.3	(6)	8.2	(5)	1.5	(1)

TABLE 13 (cont'd)

D. KF208 (*mut6/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(669)	.11	(70)	.22	(15)
21	93.7	(627)	1.72	(115)	.86	(68)
42	77.7	(520)	6.01	(318)	2.49	(141)
63	50.2	(336)	13.4	(452)	5.47	(191)
84	39.0	(261)	15.3	(401)	6.68	(180)
126	9.6	(64)	12.6	(81)	4.8	(32)
168	.75	(5)	15.9	(8)	3.8	(2)

E. KF209 (*+ / + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(716)	.10	(72)	.28	(2)
21	104	(748)	1.49	(119)	1.1	(81)
42	98.5	(705)	4.13	(298)	2.48	(177)
63	86.5	(619)	6.75	(424)	5.38	(335)
84	51.7	(370)	9.68	(362)	8.13	(302)
126	8.5	(61)	9.9	(61)	13	(82)
168	1.7	(12)	9.9	(12)	8.3	(10)

TABLE 14

The Effect of *mut6-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)		
		21 J/m^2	42 J/m^2	63 J/m^2
KF206	*mut6-1/mut6-1*	2.3 (15)[1]106[2]	5.2 (30) 94.0	7.0 (61) 70.1
KF206	*mut6-1/mut6-1*	1.4 (8) 99.5	2.4 (13) 96.3	6.3 (28) 77.8
KF206	*mut6-1/mut6-1*	2.3 (32) 101	3.3 (46) 99.3	4.1 (41) 71.5
KF207	*mut6-1/* +	.5 (5) 106	1.8 (8) 97.2	4.2 (19) 97.2
KF208	+ */mut6-1*	3.0 (19) 93.7	3.3 (17) 77.7	7.7 (26) 50.2
KF208	+ */mut6-1*	.3 (6) 107	3.5 (25) 101	4.2 (22) 75.0
KF209	+ / +	1.1 (8) 104	3.3 (23) 98.5	3.0 (18) 86.5
KF209	+ / +	.74 (8) 93.4	2.1 (19) 93.6	5.5 (37) 75.6

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

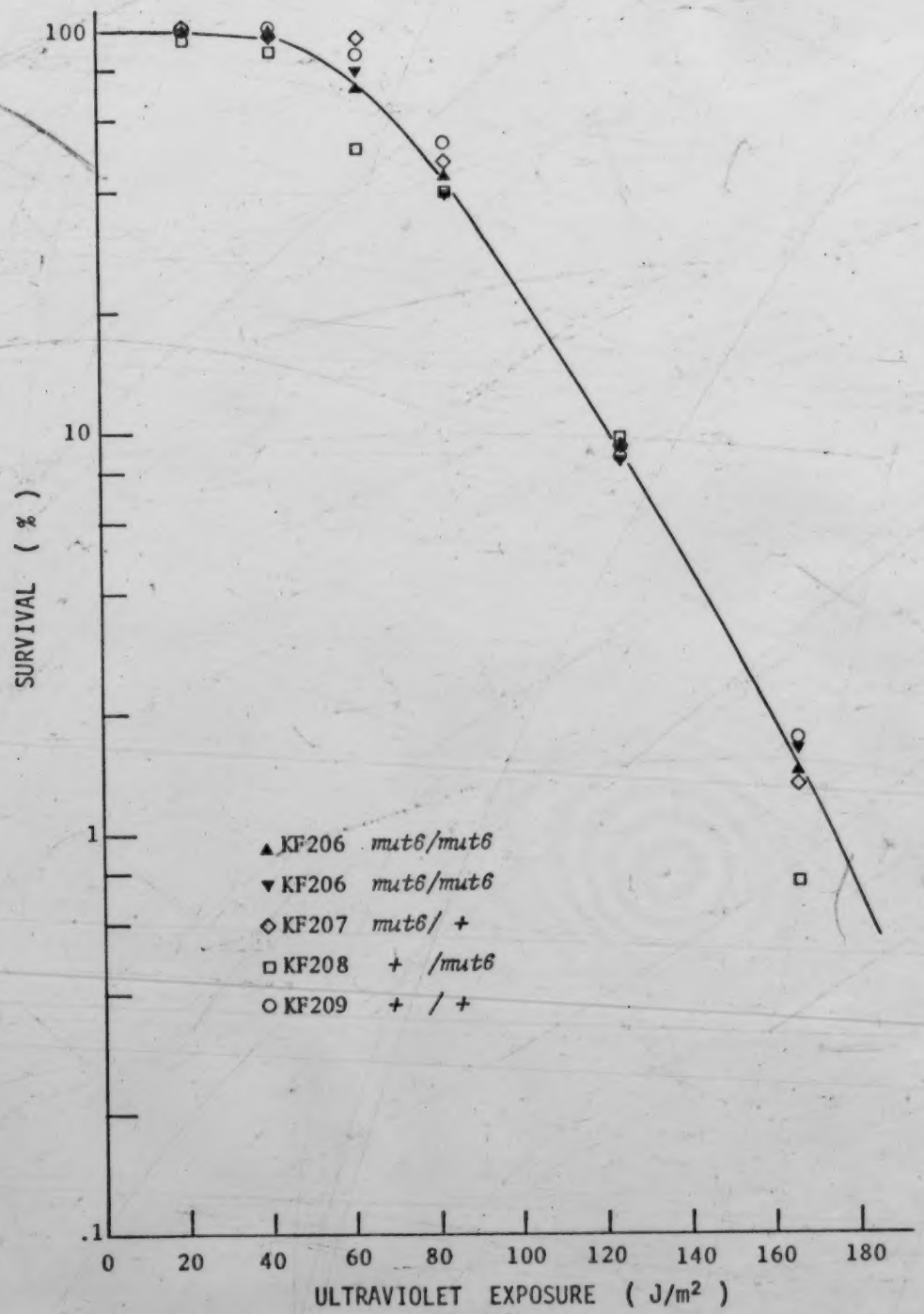

Figure 13 Survival after UV-irradiation of *mut6-1* bearing diploid strains

Only the *mut6/mut6* curve is plotted.

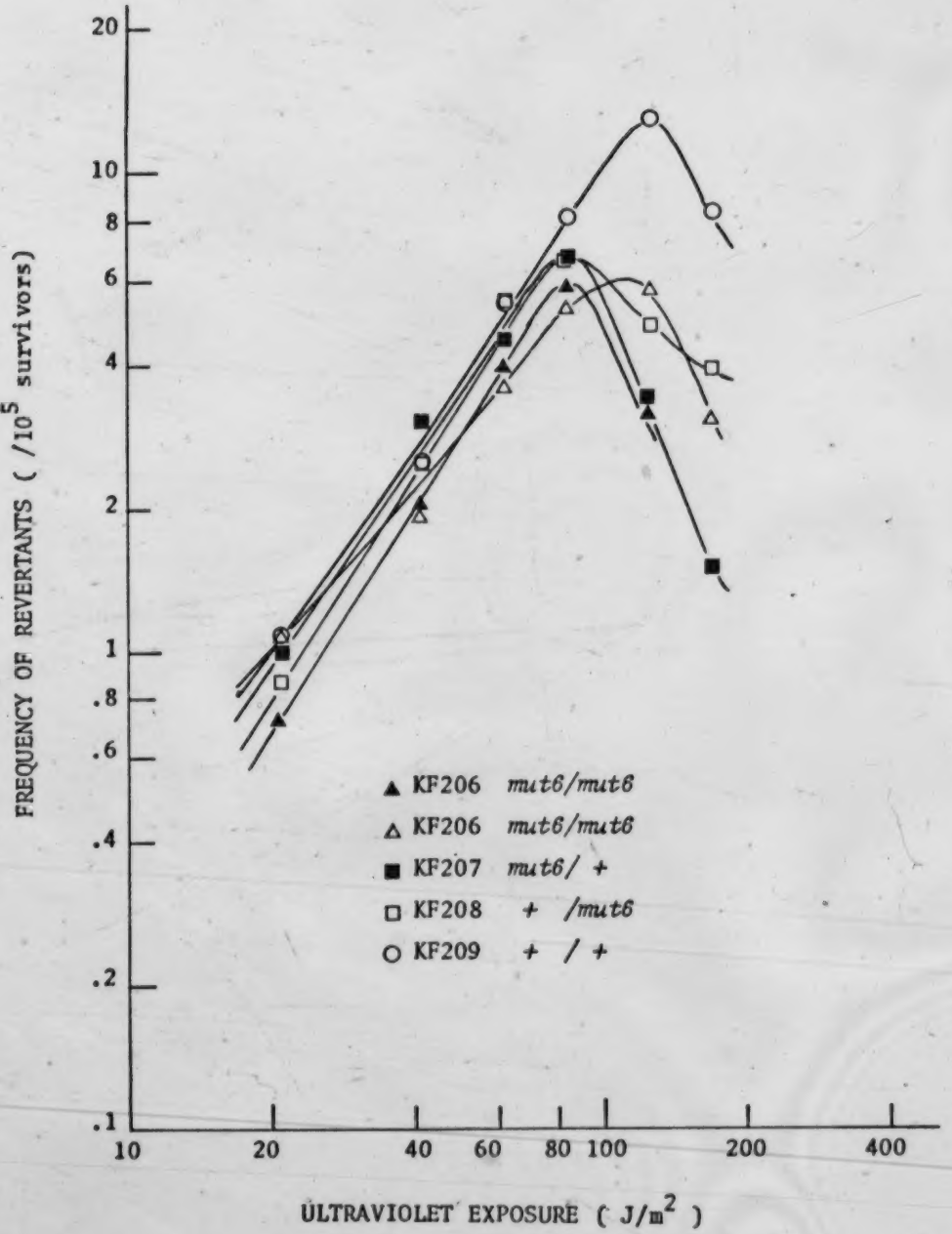

Figure 14 *lys1-1* reversion dose-response curves for *mut6-1*-bearing diploid strains

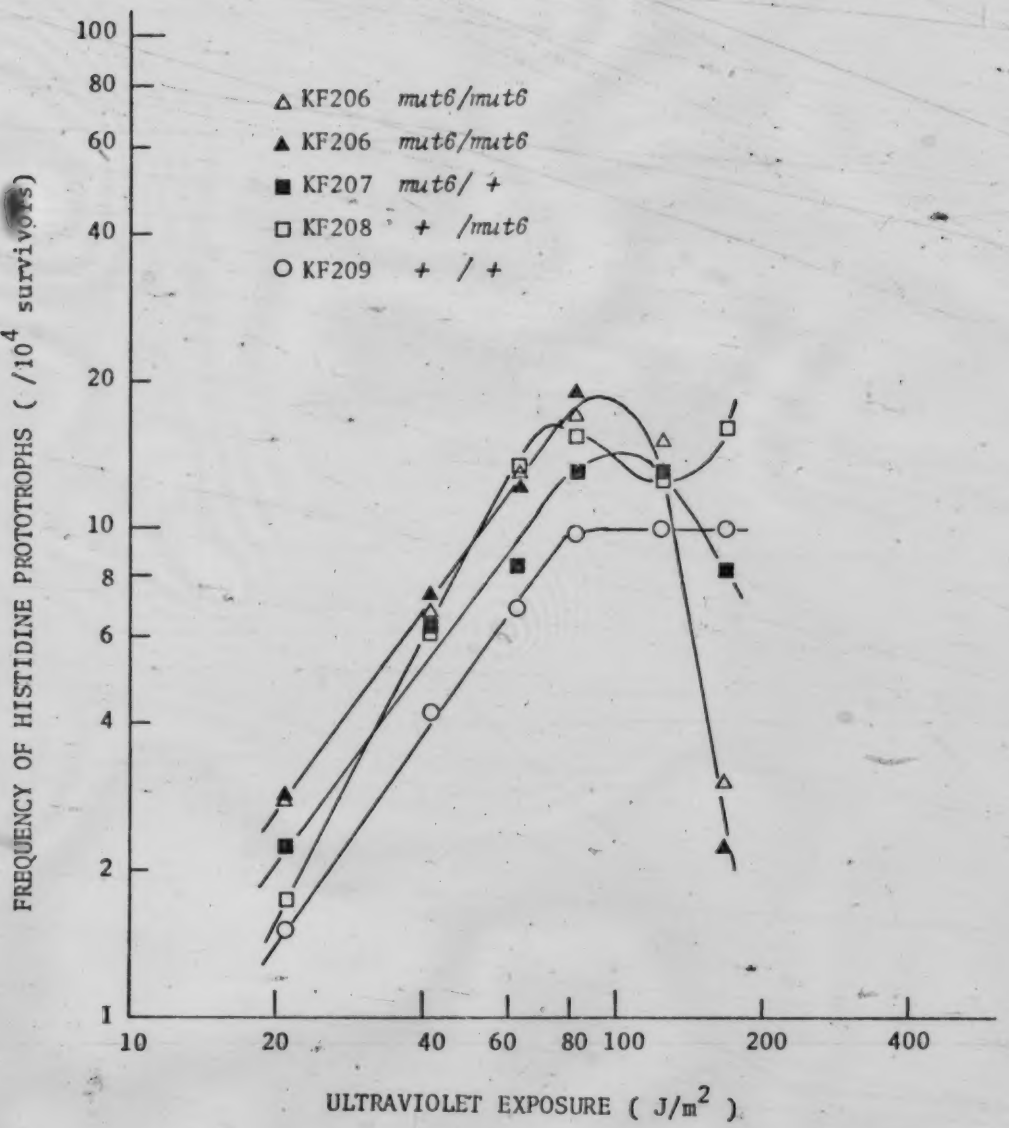

Figure 15 Dose-response curves for intragenic recombination in hetero-allelic *his 1* diploids carrying *mut6-1*

TABLE 15

The Effect of *mut9-1* on UV inactivation and Prototroph Induction[1]

A. KF210 (*mut9-1/mut9-1 ; his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(559)[4]	.08	(49)[4]	2.74	(153)[4]
21	80.7	(450)	.63	(32)	2.34	(229)
42	68.9	(385)	1.6	(63)	6.12	(341)
63	40.6	(226)	4.2	(98)	9.26	(272)
84	18.4	(103)	4.9	(61)	10.1	(131)
126	.503	(281)	7.0	(2)	15	(5)
168	.029	(16)		(0)	28	(1)

[1] Strains constructed from meiotic products of KF183

[2] All strains homozygous *lys1-1*

[3] Induction frequencies corrected for spontaneous level

[4] Colony counts on which frequencies based

TABLE 15 (cont'd)

B. KF210 (*mut9-1/mut9-1 ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(498)	.51	(253)	1.7	(86)
21	77.3	(385)	.97	(57)	1.93	(141)
42	47.2	(235)	3.3	(90)	5.93	(180)
63	20.5	(102)	3.6	(42)	7.87	(98)
84	4.4	(22)	4.5	(11)	6.0	(17)
126	.070	(35)		(0)		(0)
168	.004	(2)		(0)		(0)

C. KF211 (*mut9-1/ + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(448)	.57	(254)		(0)
21	91.7	(411)	4.08	(191)	.95	(39)
42	96.7	(433)	5.73	(273)	2.68	(116)
63	56.3	(252)	11.3	(299)	5.20	(131)
84	28.3	(127)	7.38	(101)	6.0	(76)
126	2.5	(11)	4.9	(6)	3.6	(4)

TABLE 15 (cont'd)

D. KF212 (*mut9-1/ + ; his1-1/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(453)	.40	(179)		(0)
21	92.5	(419)	3.04	(144)	1.46	(61)
42	92.7	(420)	7.17	(303)	3.60	(151)
63	76.8	(348)	10.2	(368)	6.58	(229)
84	44.2	(200)	9.70	(202)	7.55	(151)
126	5.7	(26)	3.45	(10)	5.8	(15)

E. KF213 (*+ / + ; his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(446)	.33	(147)		(0)
21	91.3	(407)	3.18	(143)	1.15	(47)
42	91.7	(409)	6.91	(296)	3.96	(162)
63	69.3	(309)	13.1	(414)	5.70	(176)
84	50.0	(223)	15.1	(343)	4.71	(105)
126	4.22	(193)	8.7	(17)	15.4	(29)
168	.39	(17)	11	(2)	11.6	(2)

TABLE 16

The Effect of *mut9-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)								
		21 J/m^2			42 J/m^2			63 J/m^2		
KF210	*mut9-1/mut9-1*				6.3	(8)[2]	56.1[3]	7.5	(8)	23.5
KF210	*mut9-1/mut9-1*	.5	(8)	80.7	1.5	(15)	68.9	4.2	(11)	40.6
KF210	*mut9-1/mut9-1*	2.1	(8)	77.3	3.4	(8)	47.2	6.8	(7)	20.5
KF211	*mut9-1/* +	1.4	(10)	92.0	2.8	(18)	86.7	6.6	(63)	65.8
KF211	*mut9-1/* +	1.5	(6)	91.7	3.5	(15)	96.7	6.4	(16)	56.3
KF212	+ */mut9-1*	1.4	(6)	92.5	2.9	(12)	92.7	5.2	(18)	76.8
KF213	+ / +	1.5	(6)	91.3	3.4	(14)	91.7	4.9	(15)	69.3
KF213	+ / +	2.0	(9)	95.0	3.8	(17)	97.8	6.5	(42)	69.3

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

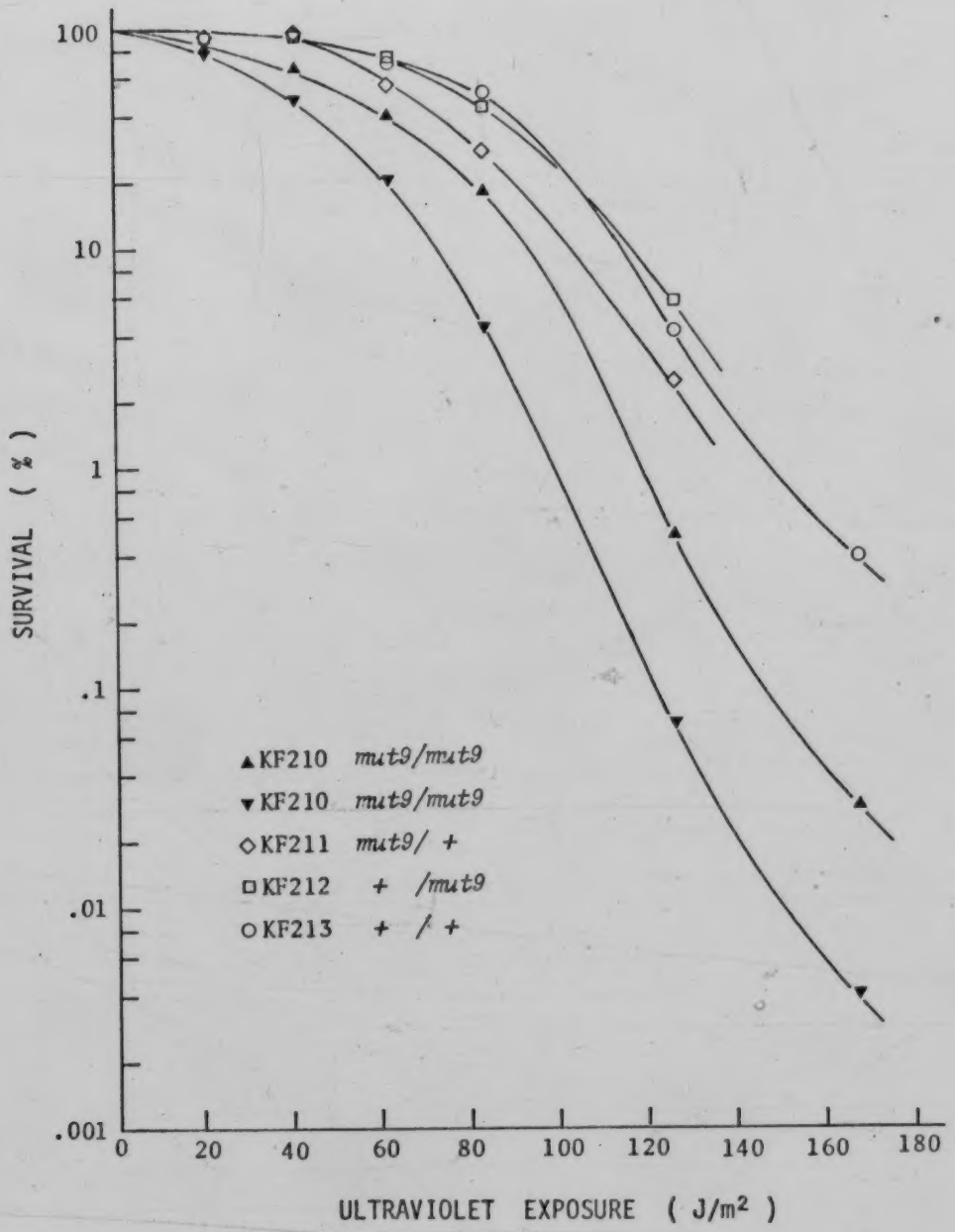

Figure 16 Survival after UV-irradiation of *mut9-1* bearing diploid strains

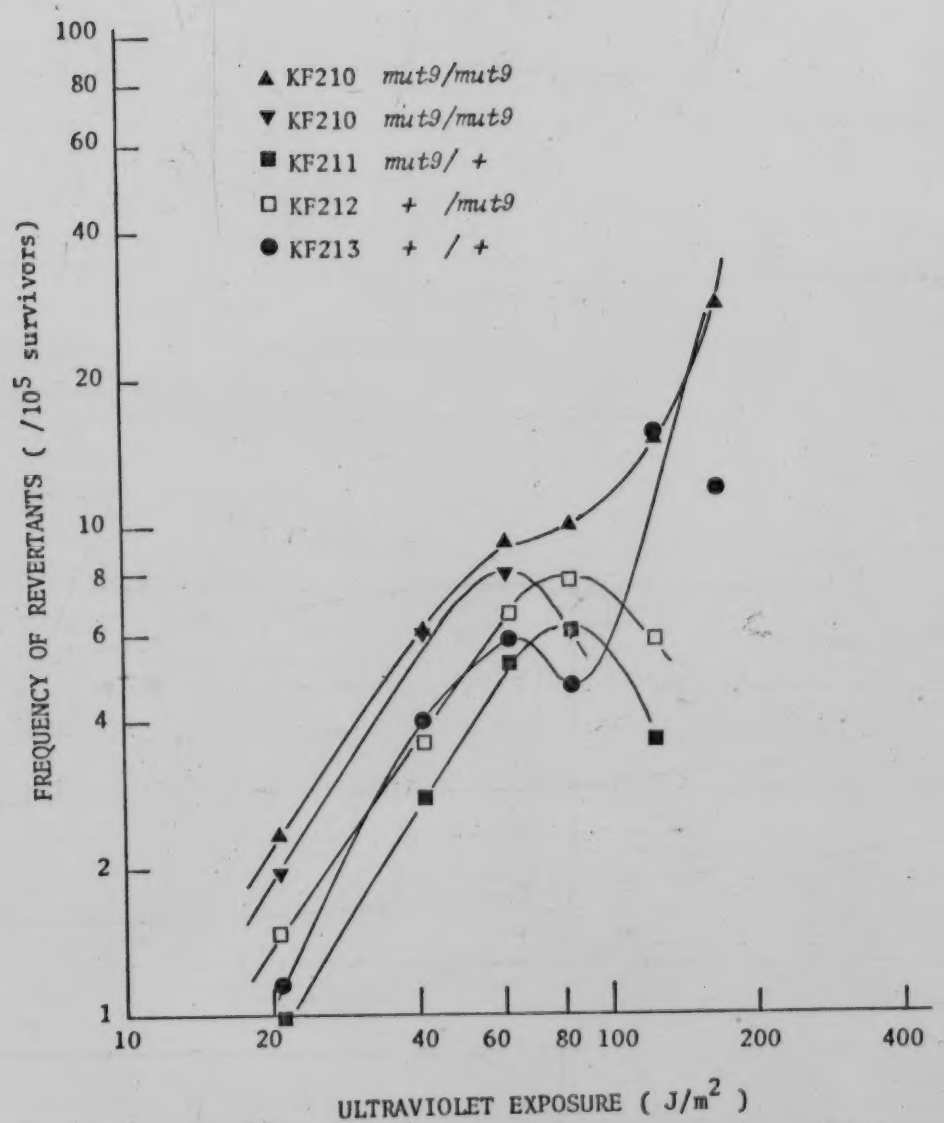

Figure 17 *lys1-1* reversion dose response curves for *mut9-1* bearing strains

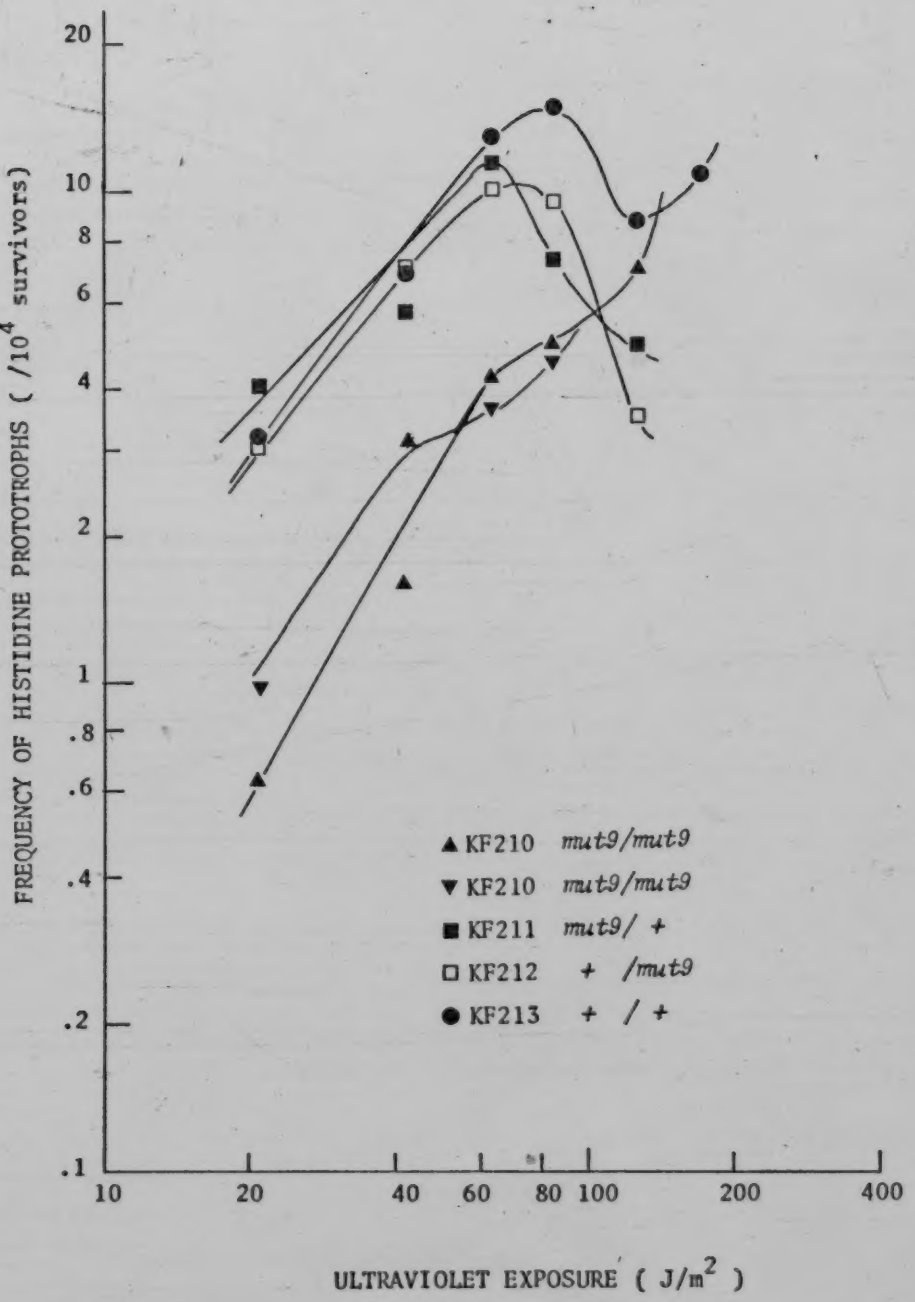

Figure 18 Dose-response curves for intragenic recombination in heteroallelic *his 1* diploids carrying *mut9-1*

mut5-1

The initial observation that *mut 5*-bearing diploids exhibited UV-induced histidine prototroph frequencies that were different from the wild type led to the collection of rather extensive data. These are contained in Tables 17-20.

The UV-inactivation curves of 2 strains of each genotype (*mut 5/mut 5*, *mut 5/ +* and *+ / +*) are plotted in Figure 19. These are typical. It will be noted that the homozygous *mut 5* strains are more sensitive to UV-light than the wild types. The curves obtained for the heterozygotes are intermediate. The inflection in the KF202 curve at 63 J/m^2 indicates the presence of a resistant sub-population of cells. Such inflections were seen more often for the homozygous *mut 5* strains.

The mutation induction dose response curves of the *mut 5* homozygous, heterozygous and related wild type strains are plotted separately in Figures 20, 21 and 22 respectively (the data are contained in Tables 17, 18 and 19 respectively). To make their comparison easier, the ranges of frequencies at all doses for the 3 sets are presented in Figure 23. At all doses the heterozygote ranges are clearly not different from the wild type. The *mut 5/mut 5* range at 21 J/m^2 encompasses that of the wild type. With increasing dose, the ranges appear to be diverging. The inflection in the wild type and heterozygote "curves" at 84-105 J/m^2 suggests that a convergence of all 3 sets is imminent.

The data on UV-induction of histidine prototrophs in *his 1*-heteroallelic, homozygous *mut 5*, heterozygous and wild type strains (from Table 17A-H, Table 18A-F and Table 19A-E respectively) are plotted separately in Figures 24, 25 and 26. The ranges of frequencies for all 3 genotypes at all doses are shown in Figure 27. While wide ranges of frequencies were obtained for all 3, it is clear that the *mut 5* homozygotes exhibit

much lower frequencies than the wild type diploids and that the heterozygotes are intermediate. Although it may misrepresent the data by obscuring the fluctuations in individual curves, in the transition region particularly, this means of presentation makes the biphasic nature of the wild type data quite clear.

The extent to which mutation was contributing to the frequencies of histidine prototrophs was investigated using strains which were homozygous for *his1-1* or *his1-315*. In such strains all prototrophs obtained should be of mutagenic origin. The results (extracted from Table 17I-L, Table 18G and H and Table 19F-I) are summarized in Table 21. They indicate that over the dose range checked, the frequencies of prototrophs in homozygous *mut 5*, heterozygous and wild type strains do not differ appreciably, and that they are in the same range as those obtained for *his 1* heteroallelic, *mut 5* homozygous strains.

No effect of *mut 5* on UV-induced homozygosis of *ade 2* is apparent in Table 20.

TABLE 17

Homozygous *mut5-1* diploids[1]: UV inactivation and prototroph induction

A. KF202 (*his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(683)[4]	.0118	(805)[4]	1.11	(757)[4]
21	94.4	(645)	.0192	(200)	.77	(1197)
42	58.1	(397)	.0972	(433)	4.18	(210)
63	11.3	(771)	.461	(364)	13.0	(109)
84	4.93	(337)	.570	(196)	20.3	(722)
105	1.60	(1096)	.910	(101)	22.2	(255)
126	.266	(1814)				
147	.0572	(391)				
168	.00630	(430)				
189	.00098	(669)				
210	.00021	(141)				

[1] Constructed from meiotic products of KF179

[2] All strains *lys1-1* homozygous

[3] Induction frequencies corrected for spontaneous levels

[4] Colony counts on which frequencies based

TABLE 17 (cont'd)

B. KF202 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(772)	.0013	(10)	.367	(283)
21	78.5	(606)	.0210	(135)	1.07	(870)
42	48.6	(751)	.112	(424)	3.68	(1595)
63	11.5	(889)	.439	(391)	15.3	(1400)
84	2.30	(1775)	1.12	(198)	34.5	(620)
105				(49)		(181)
126				(8)		(19)
147						(4)

C. KF223 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(862)	.0036	(31)	.819	(706)
21	78.9	(680)	.0246	(192)	1.11	(1315)
42	53.6	(924)	.122	(580)	3.91	(2185)
63	9.97	(859)	.831	(717)	23.2	(2065)
84	2.41	(2074)	1.90	(394)	47.5	(1001)
105				(127)		(297)
126				(27)		(81)
147				(2)		(9)

TABLE 17 (cont'd)

D. KF223 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1140)	.00304	(346)	.701	(799)
21	83.5	(952)	.0187	(207)	.629	(1262)
42	59.1	(674)	.0759	(532)	3.59	(289)
63	13.2	(1501)	.317	(481)	9.40	(152)
84	4.66	(531)	.338	(181)	13.0	(727)
105	.725	(827)	.674	(56)	21.9	(187)
126	.198	(2254)				
147	.0147	(168)				
168	.00117	(133)				
189	.00048	(547)				
210	.00023	(259)				

E. KF223 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(667)	.0048	(32)	
7	100	(670)	.0049	(65)	
14	96.7	(645)	.0142	(124)	
21	94.9	(633)	.0355	(255)	
28	80.7	(538)	.0669	(386)	
35	81.6	(544)	.0934	(534)	
42	65.8	(439)	.147	(666)	
56	27.9	(1858)	.320	(603)	
70	11.3	(1510)	.521	(397)	
84	2.70	(1798)	.785	(142)	
105	.289	(3852)			
126	.023	(3042)			

TABLE 17 (cont'd)

F. KF224 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(604)	.0075	(45)	.709	(428)
21	76.3	(461)	.0311	(178)	1.84	(1177)
42	64.9	(784)	.161	(657)	4.02	(1855)
63	20.4	(1230)	.380	(477)	15.1	(1945)
84	6.26	(3780)	.773	(295)	26.1	(1014)
105				(102)		(291)
126				(16)		(41)
147				(2)		(4)

G. KF225 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1314)	.00256	(336)	.674	(885)
21	78.0	(1025)	.0097	(126)	.576	(1179)
42	46.7	(613)	.0555	(356)	2.43	(190)
63	5.65	(743)	.363	(272)	16.1	(125)
84						
105	.845	(1110)	.384	(43)	19.8	(228)
126	.0963	(1266)				
147	.0284	(373)				
168	.0023	(300)				
189	.00055	(725)				
210	.00010	(110)				

TABLE 17 (cont'd)

H. KF225 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(562)	.0030	(17)	.856	(481)
21	81.3	(457)	.0395	(194)	1.30	(986)
42	44.1	(496)	.164	(415)	5.91	(1679)
63	21.1	(1185)	.397	(474)	13.6	(1718)
84	4.77	(2678)	.949	(255)	30.1	(831)
105				(99)		(276)
126				(20)		(37)
147				(1)		(6)
168						(1)

I. KF217 (*his1-315/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(485)	.0045	(22)	.788	(382)
21	74.6	(362)	.0372	(151)	1.91	(976)
42	40.9	(397)	.195	(395)	7.70	(1685)
63	12.0	(580)	.541	(316)	19.2	(1161)
84	3.83	(1856)	.718	(134)	22.2	(427)
105				(14)		(65)
126						(6)

TABLE 17 (cont'd)

J. KF217 (*his1-315/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1170)	.0161	(1880)	.420	(491)
21	84.6	(990)	.0164	(322)	.510	(921)
42	55.4	(648)	.0620	(506)	2.94	(218)
63	10.4	(1211)	.305	(389)	7.92	(101)
84	4.47	(523)	.345	(189)	5.93	(332)
105	1.32	(1546)	.521	(83)	13.5	(215)

K. KF226 (*his1-1/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1722)	.00203	(350)	.437	(753)
21	81.5	(1403)	.00752	(134)	.265	(985)
42	54.3	(935)	.0315	(313)	5.85	(197)
63	4.92	(847)	.408	(347)	8.65	(77)
84	1.78	(307)	.490	(151)	13.3	(422)
105	.305	(526)	1.18	(62)	17.1	(92)
126	.0169	(291)				
147	.00134	(23)				
168	.000197	(34)				
189	.000083	(142)				
210	.000069	(119)				

TABLE 17 (cont'd)

L. KF220 (*his1-1/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(851)	.0001	(1)	.157	(136)
21	70.3	(598)	.016	(94)	.973	(679)
42	45.2	(770)	.057	(219)	3.70	(1486)
63	9.69	(825)	.240	(198)	18.4	(1534)
84	1.32	(1122)	.51	(57)	58.2	(655)
105				(16)		(134)
126				(5)		(18)

TABLE 18

Heterozygous *mut5-1* diploids[1]: UV inactivation and prototroph induction

A. KF204 (*his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(931)[4]	.016	(30)[4]	.024	(22)[4]
21	87.6	(816)	.574	(48)	1.52	(251)
42	81.2	(756)	1.96	(150)	4.56	(346)
63	59.6	(1109)	3.64	(203)	7.41	(824)
84	16.2	(1510)	8.32	(126)	18.4	(2780)
105	6.04	(1124)	9.4	(53)	28.3	(1589)
126	1.56	(1450)	8.3	(24)	54.4	(789)
147	.537	(1000)	5.0	(25)	44.0	(220)
168	.0821	(1529)	10	(8)	80	(61)
189	.0144	(1338)	81.4	(109)	130	(18)
210	.0060	(557)	65	(36)	130	(7)

[1] Constructed from meiotic products of KF179

[2] All strains *lys1-1* homozygous

[3] Induction frequencies corrected for spontaneous levels

[4] Colony counts on which frequencies based

TABLE 18 (cont'd)

B. KF204 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(2637)	.226	(5960)*	.0090	(237)
21	97.1	(1280)	.195	(1079)		
42	85.9	(1133)	.743	(2196)	1.65	(376)
63	59.2	(780)	1.28	(2360)	3.02	(472)
84	17.3	(457)	2.12	(1073)	5.00	(229)
105	2.27	(599)	10.4	(634)	15.2	(91)
126	.990	(261)	14.8	(3920)*	23.1	(603)
147	.0974	(257)	49.3	(1266)	94.2	(242)
168	.0316	(834)				
189	.0033	(869)				

C. KF203 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1708)	.0650	(1110)	.0218	(373)
21	101	(1719)	.140	(352)		
42	88.3	(1508)	.412	(719)	2.78	(422)
63	57.0	(974)	.782	(825)	4.12	(403)
84	15.5	(265)	1.64	(454)	8.85	(235)
105	2.58	(440)	3.13	(141)	11.6	(51)
126	.855	(146)	6.70	(988)	28.6	(418)
147	.0872	(149)	34.9	(520)	91.3	(136)

* Estimate

TABLE 18 (cont'd)

D. KF203 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)
0	100	(466)	.183	(1705)	
21	90.1	(420)	.786	(407)	
42	91.4	(426)	2.23	(1027)	
63	43.6	(406)	6.42	(1340)	
84	11.0	(511)	16.5	(1710)	
105	2.41	(1123)	22.6	(5118)	
126	.418	(1949)	68.1	(2664)	

E. KF203 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(844)	.020	(33)	.024	(20)
21	106	(893)	.44	(41)	1.91	(344)
42	96.7	(816)	.78	(65)	4.89	(401)
63	61.2	(1034)	1.4	(73)	8.31	(861)
84	18.4	(1554)	1.8	(28)	17.1	(2658)
105	4.67	(788)	7.3	(29)	34.2	(1346)
126	.944	(797)	5	(8)	83.3	(664)
147	.304	(513)	2	(5)	44.1	(113)
168	.0404	(682)	3	(1)	79	(27)
189	.00805	(679)	37	(25)	130	(9)
210	.00351	(296)	30	(8)		

TABLE 18 (cont'd)

F. KF203 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1320)	.0386	(510)	.0033	
21	93.2	(1230)	.229	(330)	1.42	
42	88.3	(1165)	.394	(101)	3.23	
63	58.6	(1548)	.265	(47)	4.82	
84	17.8	(4698)	.463	(118)	9.35	

G. KF219 (*his1-315/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1727)	.00014	(1)	.0072	(25)
21	89.0	(1537)	.0097	(3)	.50	(312)
42	83.4	(1441)	.024	(7)	1.27	(370)
63	40.6	(1404)	.057	(8)	3.10	(872)
105	3.56	(1229)			10.7	(1310)
126	.325	(1122)			50.4	(566)
147	.112	(775)			35.1	(136)
168	.0219	(1510)			15	(11)
189	.00511	(1764)			30	(5)

TABLE 18 (cont'd)

H. KF222 (*his1-1/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(821)		(0)	.057	(47)
21	84.0	(690)	.03	(2)	1.84	(262)
42	80.8	(663)	.15	(10)	4.06	(273)
63	55.4	(909)	.24	(11)	4.65	(428)
84	25.1	(1030)	.4	(8)	9.50	(1970)
105	6.29	(1032)			28.7	(1485)
126	2.61	(1073)	.5	(2)	29.0	(625)
147	.584	(959)			60.8	(292)
168	.134	(1101)			69.4	(73)
189	.0332	(1361)			110	(30)

TABLE 19

Wild-type diploids[1]: UV inactivation and prototroph induction

A. KF205 (*his1-315/his1-1*)[2]

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)[3]		Frequency of lysine revertants ($/10^5$ survivors)[3]	
0	100	(2502)[4]	.0263	(657)[4]	3.30	(8256)*[4]
21	98.8	(2472)	.542	(1404)	1.04	(1074)
42	89.8	(2246)	1.98	(451)	1.69	(1120)
63	71.8	(1796)	3.39	(615)	2.01	(954)
84	42.5	(1063)	3.42	(367)	2.22	(587)
105	7.90	(1976)	8.79	(1742)	13.9	(3404)
126	2.66	(665)	9.79	(653)	17.4	(1444)
147	.352	(880)	41.0	(1804)		
168	.112	(2794)	67.4	(1884)		
189	.0200	(5000)*				
210	.00578	(1445)				

* estimate

[1] Constructed from meiotic products of KF179

[2] All strains *lys1-1* homozygous

[3] Induction frequencies corrected for spontaneous levels

[4] Colony counts on which frequencies based

TABLE 19 (cont'd)

B. KF205 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(314)	.0379	(238)		
21	112	(352)	1.72	(619)		
42	98.1	(308)	3.62	(1126)		
63	75.8	(476)	6.16	(295)		
84	33.8	(211)	4.26	(454)		
105	7.74	(243)	6.65	(325)		
126	2.05	(129)	7.60	(493)		
147	.43	(134)	17.5	(468)		
168	.024	(15)	75.3	(113)		
189	.0032	(10)	135	(27)		
210	.0010	(31)	177	(11)		

C. KF231 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidien prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1908)	.372	(7100)*	.0052	(10)
21	93.5	(1784)	1.07	(2548)	.780	(140)
42	95.2	(1816)	5.90	(1139)	1.92	(349)
63	86.0	(1640)	5.25	(922)	2.29	(376)
84	43.2	(824)	9.83	(843)	2.31	(190)
105	7.74	(148)	13.2	(2014)	9.62	(1422)
126	2.74	(523)	15.5	(831)	13.9	(726)
147	1.27	(243)	16.6	(4132)*		
168	.0508	(970)	157	(1522)		
189	.0245	(468)				
210	.0056	(106)				

* estimate

TABLE 19 (cont'd)

D. KF227 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(635)	.167	(212)	.060	(19)
21	96.2	(611)	2.43	(159)	2.91	(907)
42	96.5	(613)	5.18	(328)	5.94	(1841)
63	78.3	(497)	10.1	(510)	8.44	(2118)
84	45.5	(289)	10.7	(314)	14.9	(2168)
105	15.8	(201)	15.4	(157)	25.7	(1298)
126	4.19	(266)	14.5	(78)	61.3	(817)
147	.667	(423)	40.9	(174)	139	(295)
168	.184	(234)	65	(49)	152	(89)
189	.0200	(128)	125	(32)	156	(10)
210	.0028	(69)	767	(138)	348	(6)

E. KF229 (*his1-315/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1853)	.0132	(244)	.098	(181)
21	94.0	(1741)	.516	(921)	.367	(81)
42	93.6	(1735)	1.11	(197)	1.14	(215)
63	74.7	(1384)	2.92	(406)	2.37	(329)
84	48.4	(897)	5.80	(521)	3.86	(347)
105	20.6	(382)	10.0	(382)	5.48	(209)
126	4.53	(840)	36.8	(309)	21.5	(1808)
147	2.35	(436)				

TABLE 19 (cont'd)

F. KF218 (*his1-315/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1542)	.00534	(824)	.055	(85)
21	97.4	(1502)	.0135	(283)	.757	(122)
42	79.6	(1227)	.0508	(688)	2.17	(274)
63	69.0	(1064)	.0880	(993)	3.22	(349)
84	43.3	(668)	.197	(1350)	3.85	(261)
105	16.5	(1273)	.446	(1148)	9.32	(1198)
126	7.54	(1163)			13.6	(1587)
147	.949	(1464)				

G. KF218 (*his1-315/his1-315*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(1375)		(0)	.019	(26)
21	93.2	(1282)	.02	(3)	1.52	(395)
42	85.1	(1170)	.07	(8)	3.77	(444)
63	55.6	(764)	.24	(18)	6.54	(501)
84	24.8	(681)	.29	(10)	9.73	(332)
105	9.20	(253)	.2	(3)	19.7	(2497)
126	2.68	(368)	.5	(4)	37.3	(2748)
147	.476	(131)	1	(7)	107	(1404)
168	.105	(144)	.7	(2)	226	(652)
189	.0103	(141)	9.9	(14)	752	(212)
210	.00436	(60)	5	(3)	430	(51)

TABLE 19 (cont'd)

H. KF221 (*his1-1/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(840)	.00004	(3)	.064	(54)
21	94.4	(793)	.012	(92)	1.36	(113)
42	87.7	(737)	.0157	(1159)	2.88	(217)
63	78.6	(660)	.0980	(647)	6.20	(413)
84	50.6	(425)	.223	(947)	10.4	(444)
105	25.7	(1081)	.451	(975)	18.2	(985)
126	9.93	(834)			44.6	(930)
147	4.23	(953)				
168	.548	(575)				

I. KF221 (*his1-1/his1-1*)

Dose (J/m^2)	Survival (%)		Frequency of histidine prototrophs ($/10^4$ survivors)		Frequency of lysine revertants ($/10^5$ survivors)	
0	100	(620)		(0)	.026	(16)
21	95.2	(590)	.07	(4)	1.93	(231)
42	80.8	(501)	.22	(11)	6.24	(314)
63	57.9	(359)	.39	(14)	10.5	(377)
84	24.5	(304)	.5	(7)	12.6	(192)
105	4.8	(59)	2	(5)	59.3	(1749)
126	1.1	(71)			160	(2265)
147	.37	(46)			225	(1033)
168	.052	(32)			509	(326)
189	.0085	(53)			1070	(113)
210	.002	(9)			2100	(38)

TABLE 20

The Effect of *mut5-1* on UV-induced Homozygosis of *ade 2*

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)					
		21 J/m^2			42 J/m^2		
KF202	*mut5-1/mut5-1*	1.0	(11)[2]	94.4[3]	3.6	(17)	58.1
KF223	*mut5-1/mut5-1*	1.6	(26)	83.5	6.0	(48)	59.1
KF225	*mut5-1/mut5-1*	.8	(15)	78.0	4.5	(32)	46.7
KF226	*mut5-1/mut5-1*	1.0	(22)	81.5	3.6	(39)	54.3
KF207	*mut5-1/* +	2.6	(2)	101	7.0	(5)	94.7
KF207	*mut5-1/* +	.5	(2)	90.1	1.6	(7)	91.4
KF205	+ / +	.6	(1)	102	4.8	(8)	103
KF205	+ / +	1.1	(4)	112	2.9	(9)	98.1
KF205	+ / +	1.2	(30)	98.8	3.2	(72)	89.8
KF227	+ / +	1.0	(18)	93.5	2.3	(43)	94.5
KF227	+ / +	.8	(5)	96.2	1.8	(11)	96.5
KF231	+ / +	2.5	(24)	99.1	4.4	(42)	101
KF231	+ / +	1.1	(20)	93.5	3.7	(67)	95.2

Strain	Mutator genotype	Frequency of *ade 2* homozygotes[1] (%)		
		63 J/m^2		
KF202	*mut5-1/mut5-1*	5.3	(46)	11.3
KF223	*mut5-1/mut5-1*	5.4	(81)	13.2
KF225	*mut5-1/mut5-1*	11	(91)	5.65
KF226	*mut5-1/mut5-1*	6.2	(58)	4.92
KF207	*mut5-1/* +	5.4	(2)	65.3
KF207	*mut5-1/* +	5.7	(29)	43.6
KF205	+ / +	5.4	(7)	79.6
KF205	+ / +	5.3	(25)	75.8
KF205	+ / +	5.5	(99)	71.8
KF227	+ / +	4.1	(73)	89.0
KF227	+ / +	6.2	(31)	78.3
KF231	+ / +	9.6	(64)	69.9
KF231	+ / +	4.3	(35)	86.0

[1] As indicated by red

[2] Number of sectors on which frequency based

[3] Percent survival

TABLE 21

UV-induction of histidine prototrophs in homozygous *his1-315* or *his1-1*, *mut 5*-bearing diploid strains

A. *his1-315* homozygous strains

Dose (J/m^2)	Frequency of histidine prototrophs ($/10^4$ survivors)				
	mut5/mut5		*mut5/ +*	*+ / +*	
	KF217	KF217	KF219	KF218	KF218
21	.0372	.0160	.01	.0135	.02
42	.195	.062	.02	.0508	.07
63	.541	.305	.06	.0880	.24
84	.718	.345		.197	.29
105		.521		.446	.2
126					.5

B. *his1-1* homozygous strains

Dose (J/m^2)	Frequency of histidine prototrophs ($/10^4$ survivors)				
	mut5/mut5		*mut5/ +*	*+ / +*	
	KF226	KF220	KF222	KF221	KF221
21	.0075	.016	.03	.012	.07
42	.0315	.057	.15	.0157	.22
63	.408	.240	.24	.0980	.39
84	.490	.51	.4	.223	.5
105	1.18			.451	2
126			.5		

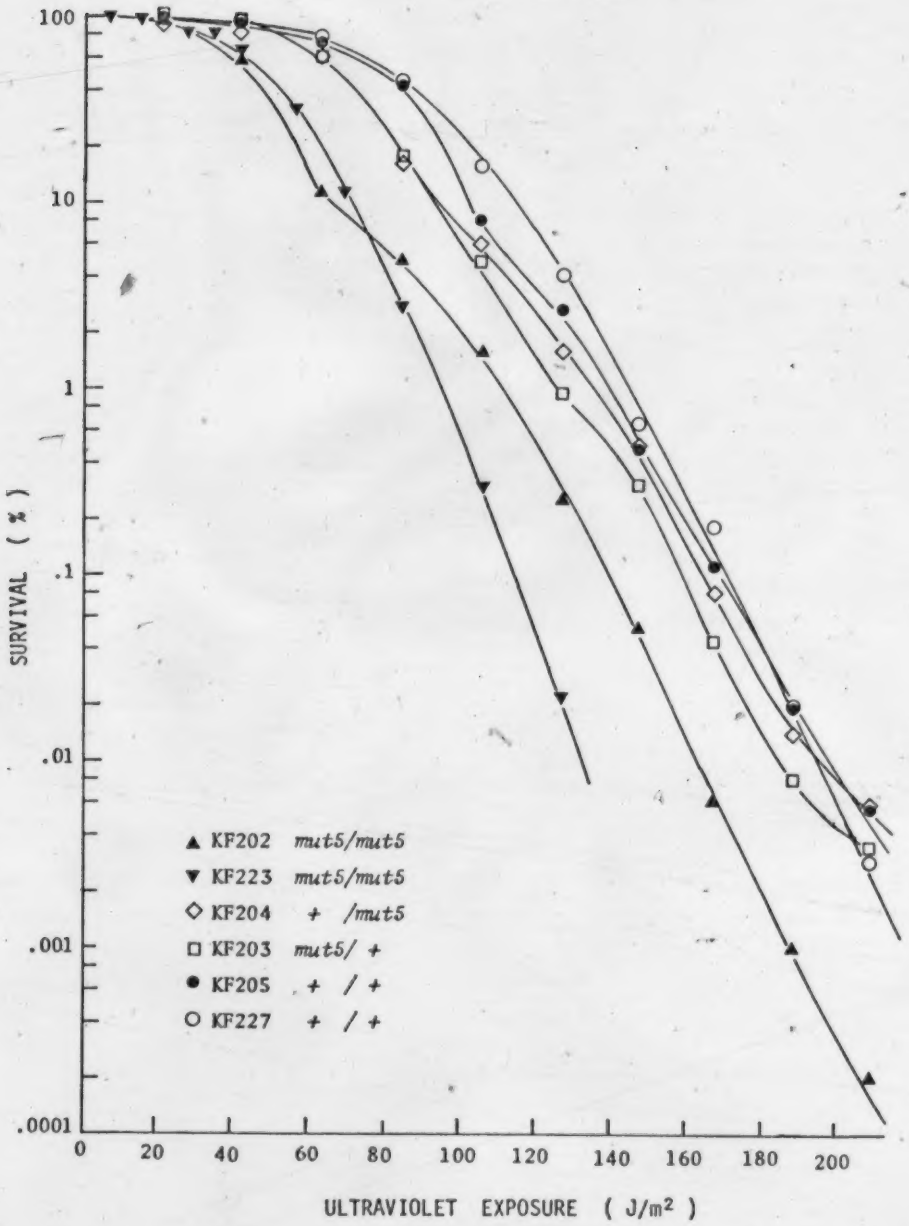

Figure 19 Survival after UV-irradiation of *mut5-1* bearing diploid strains

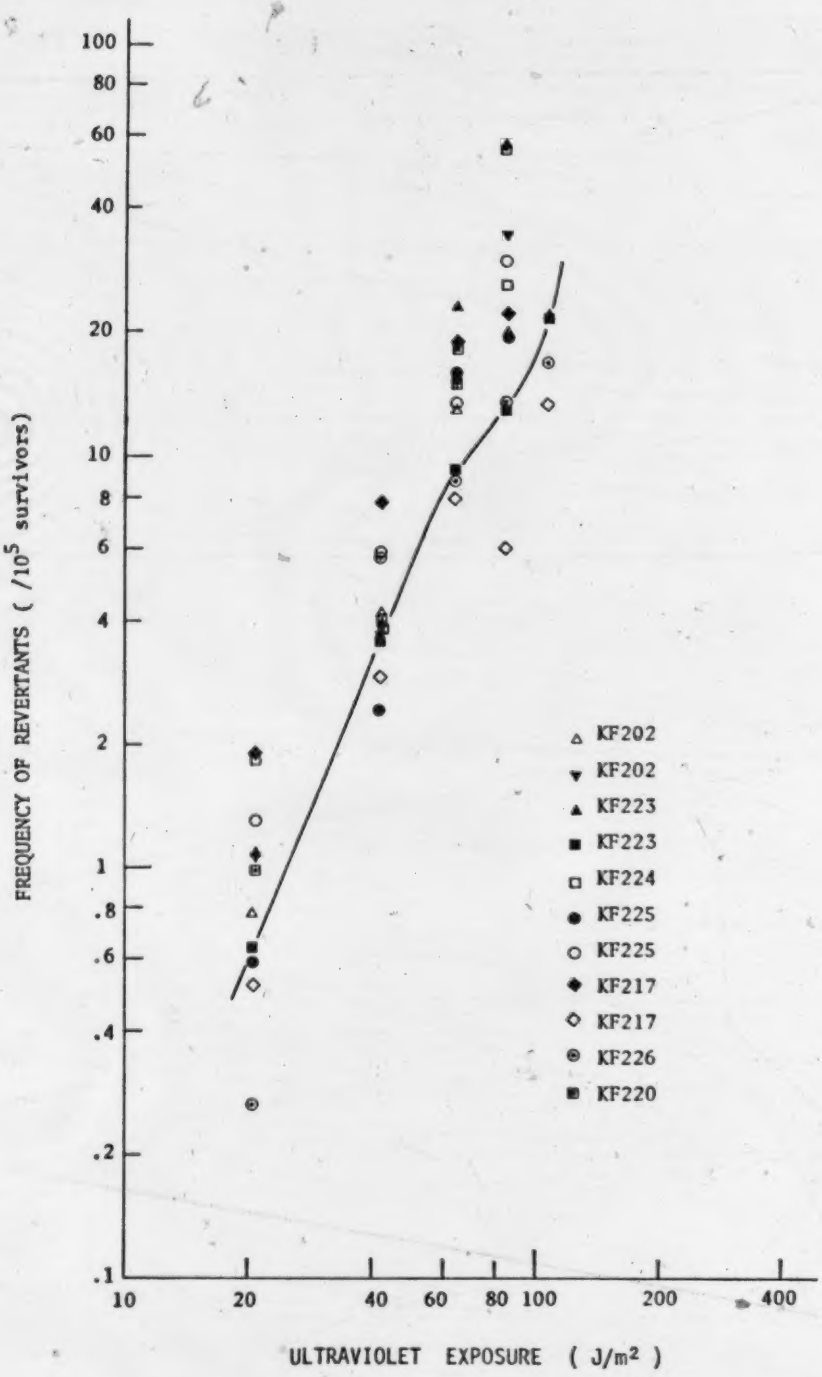

Figure 20 *lys1-1* reversion dose response data for homozygous *mut5-1* diploids. In the interests of clarity only a single curve is plotted.

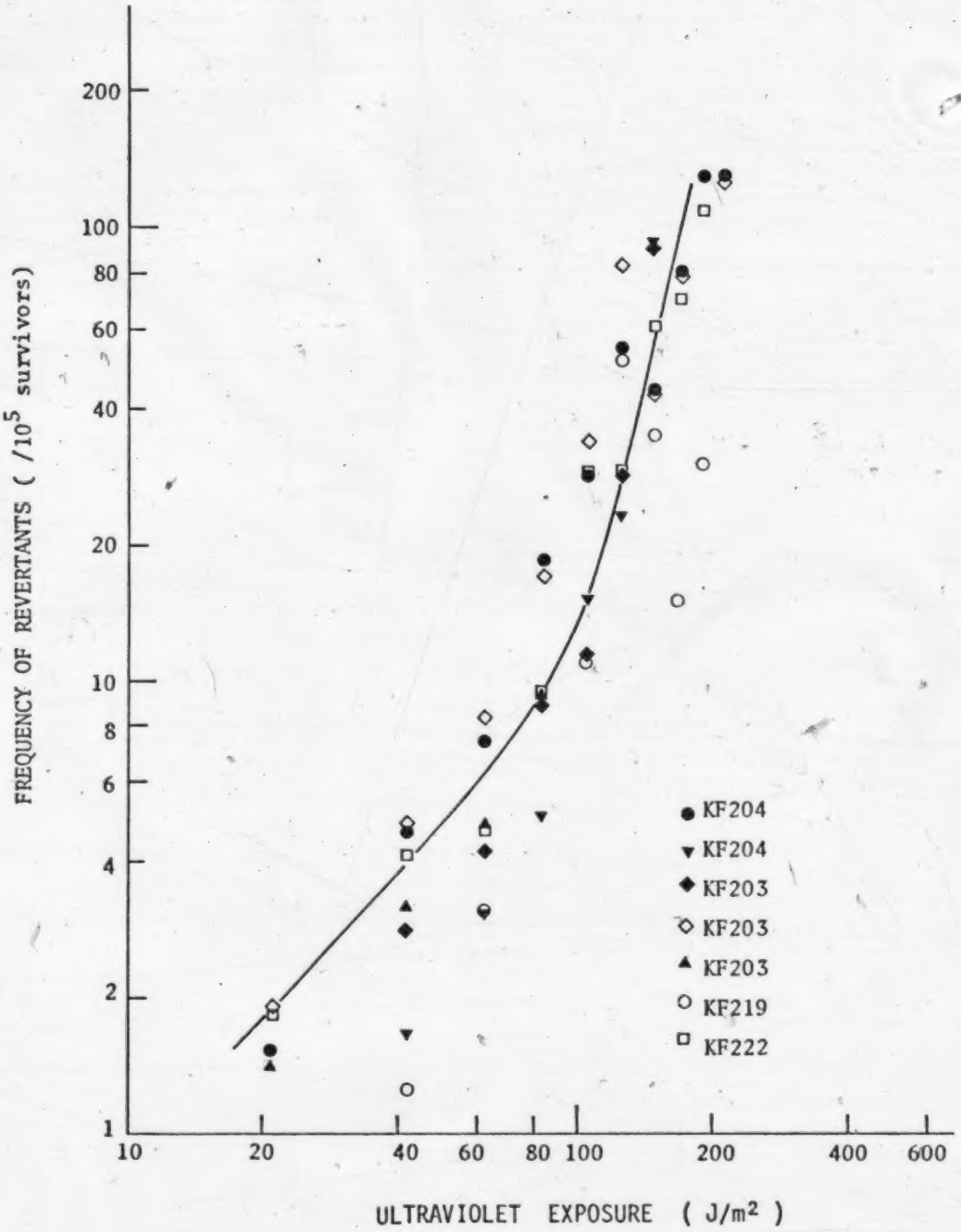

Figure 21 *lys1-1* reversion dose response data for heterozygous *mut5-1* diploids. A single reference curve is plotted.

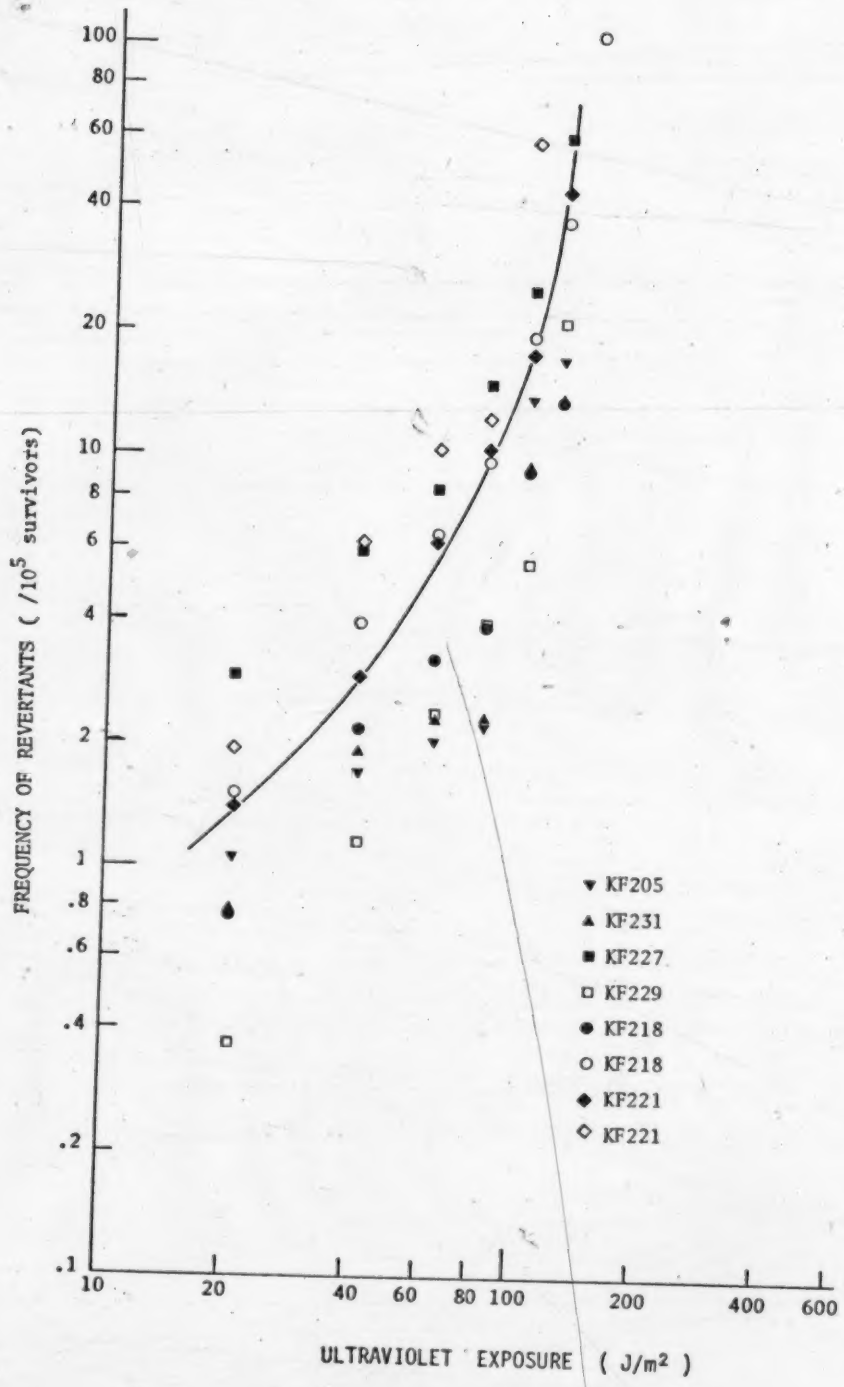

Figure 22 *lys1-1* reversion dose-response curves for wild type diploids. A single reference curve is plotted.

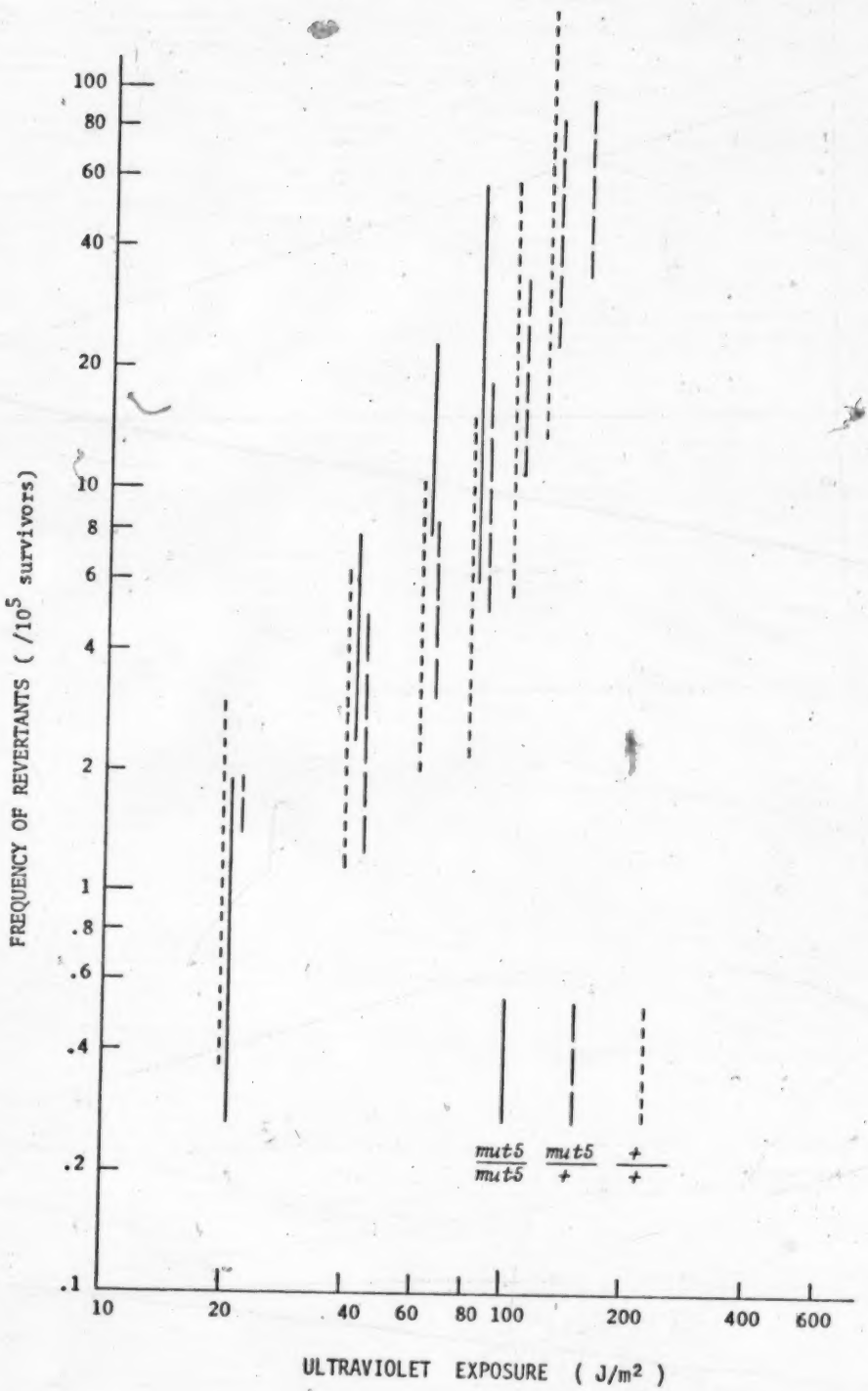

Figure 23 Ranges of induced revertant frequencies for *mut5/mut5*, *mut5/ +* and *+ / +* diploid strains

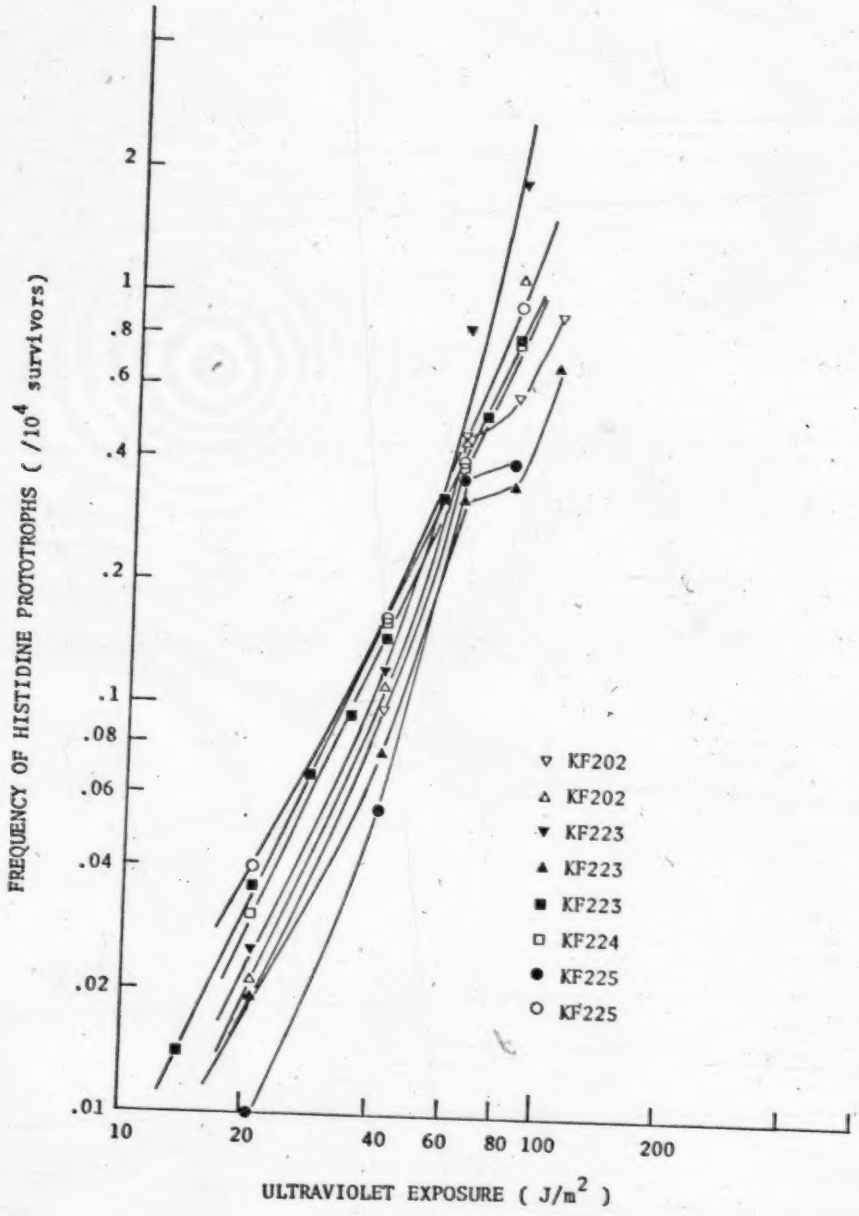

Figure 24 Dose-response curves for histidine prototroph production in *mut5-1* homozygous diploids

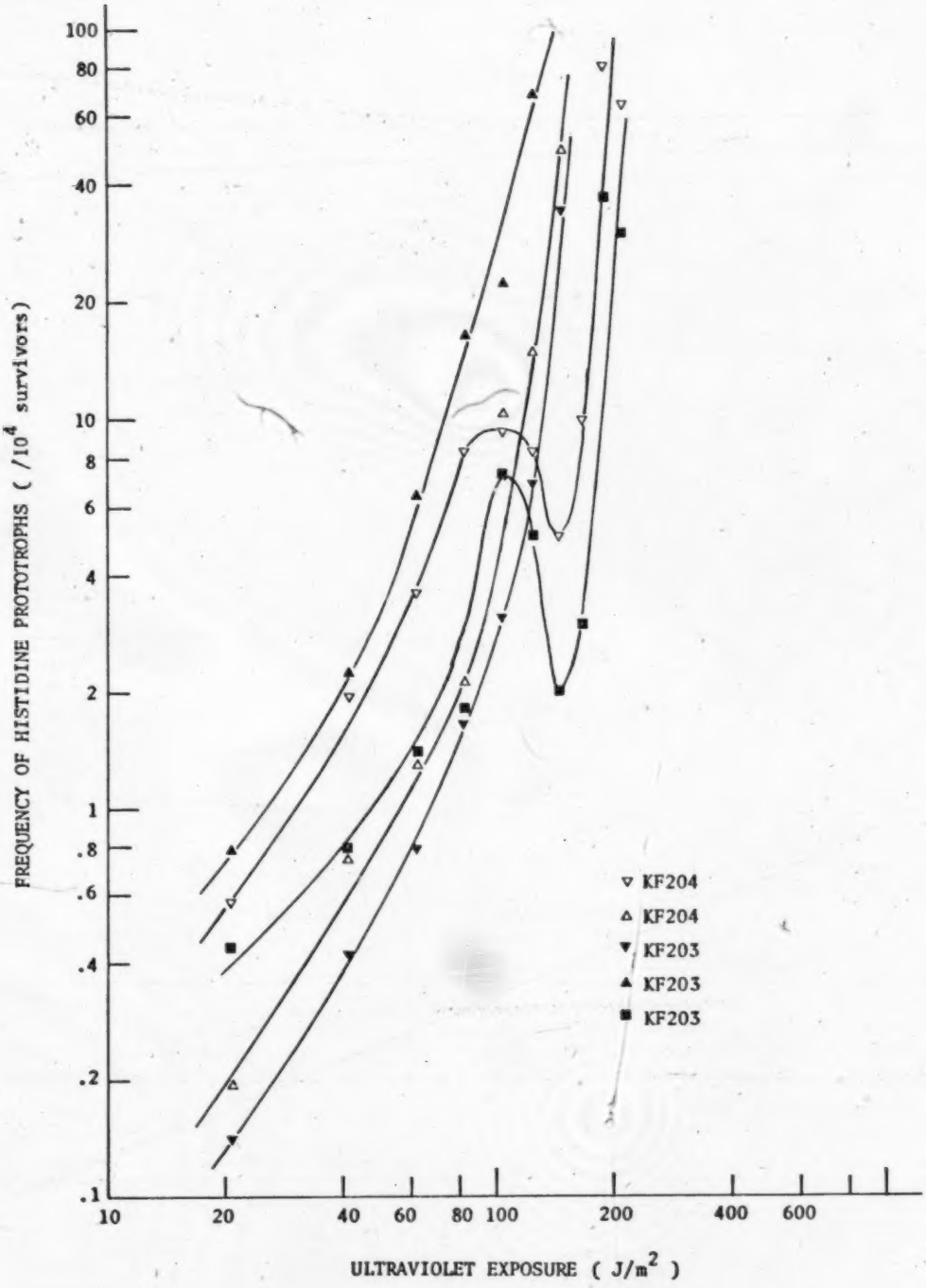

Figure 25 Dose-response curves for intragenic recombination in heteroallelic *his 1*, heterozygous *mut5-1* diploids

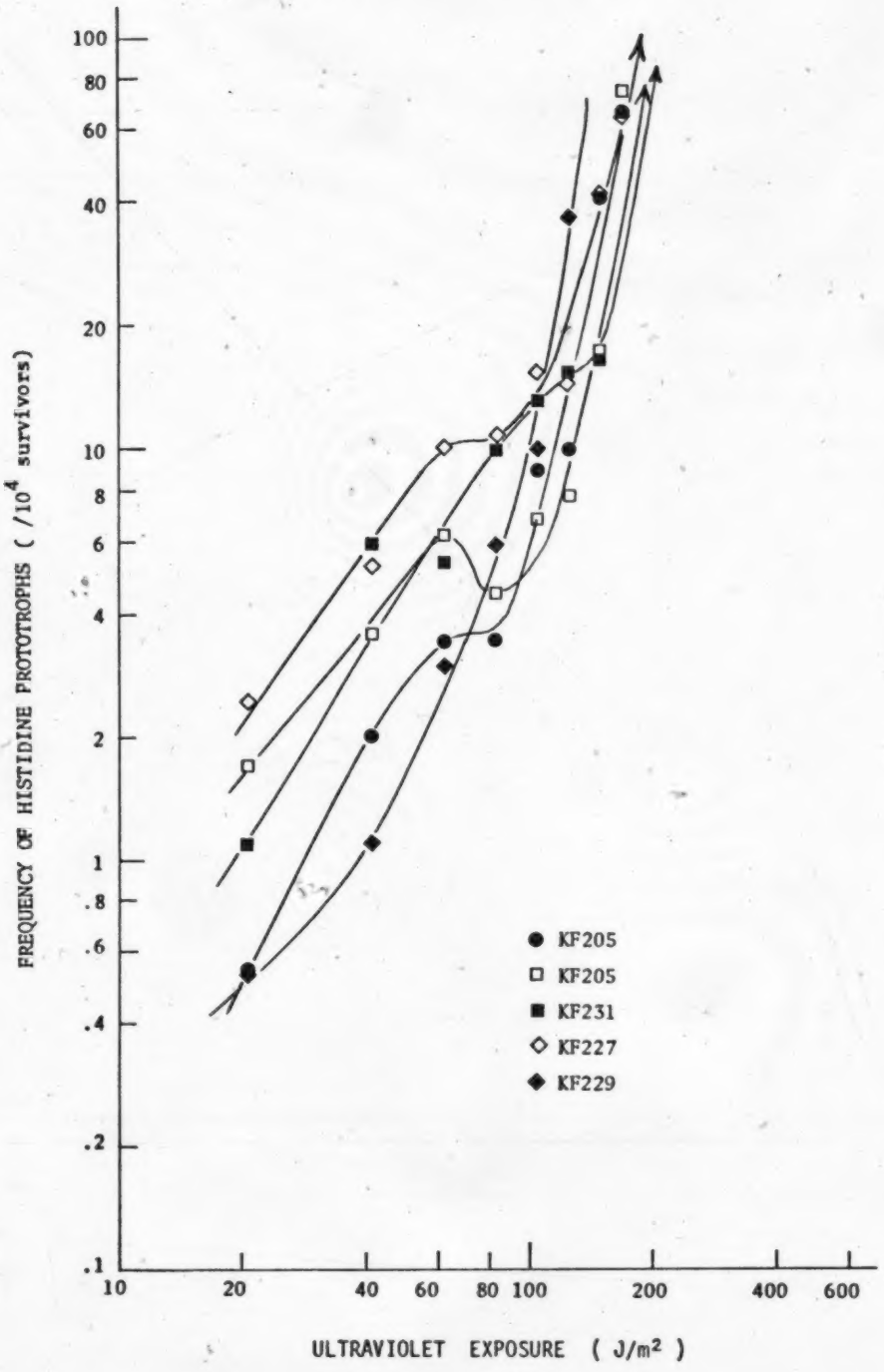

Figure 26 Dose-response curves for intragenic recombination in heteroallelic *his 1*, non-mutator diploids

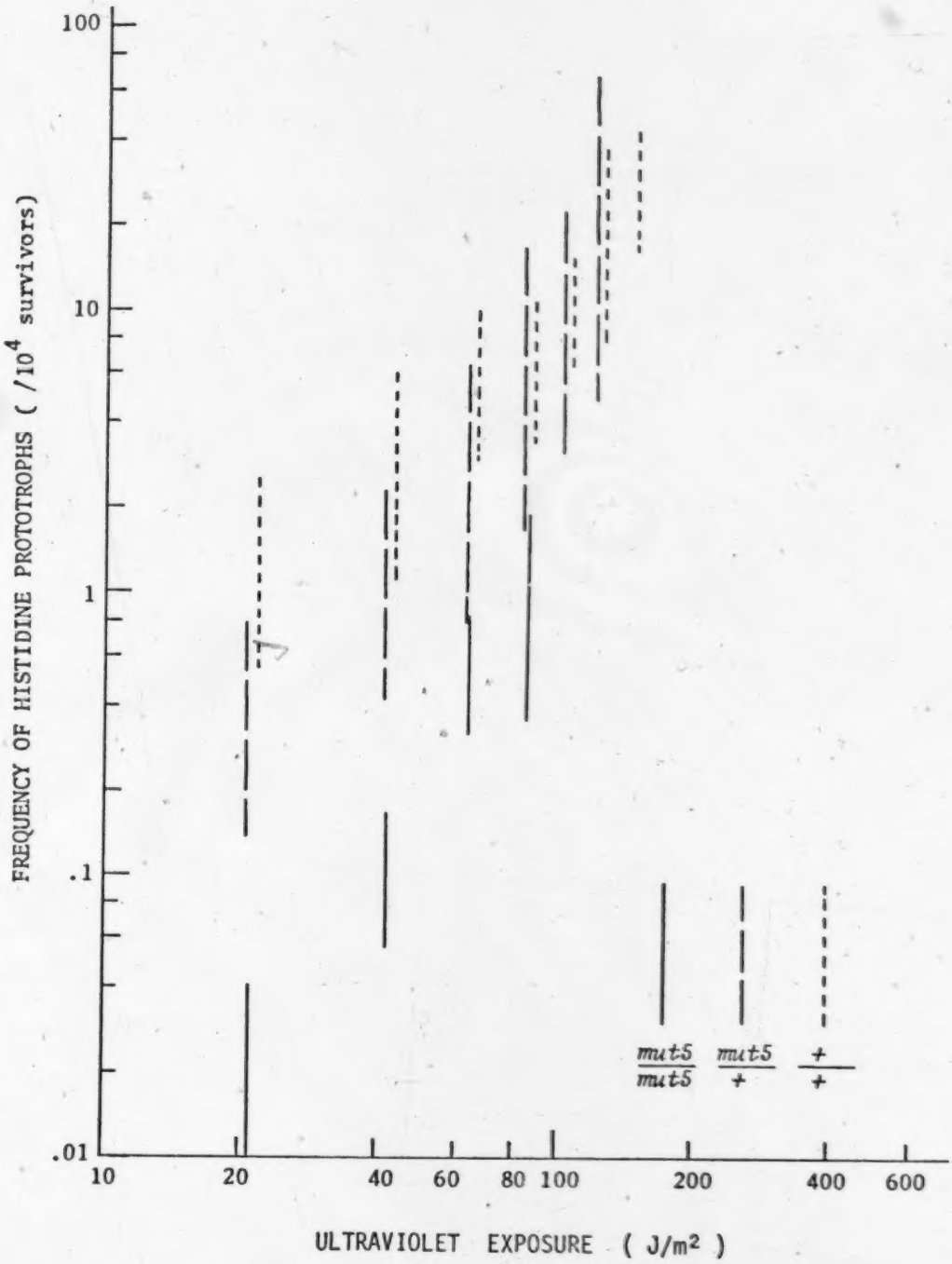

Figure 27 Ranges of induced histidine prototroph frequencies for *mut5/mut5*, *mut5/ +* and *+ / +* diploid strains

Further Characterization of *mut5-1*

mut5-1, an allele of *RAD 51*

The phenotype of *mut5-1* mutants, UV- and γ-ray sensitivity and deficiency in UV-induced intragenic recombination, is like that of some of the mutants of genes in *RAD 18* and *RAD 51* epistasis groups. The observation of S.-K. Quah (unpublished results) that *mut 5* was linked to *his 1* immediately ruled out all the X-ray sensitivity loci which had already been mapped. Thus, *rev 2* (Lemontt, 1971a), *rad 52*, *rad 55* (Mortimer and Hawthorne, 1973), *rad 6*, *rad 18*, *rad 56* and *rad 57* (Game and Mortimer, 1974) were eliminated. Only 8 remained (*rads 8*, *9*, *50*, *51*, *53* and *54* and *revs 1* and *3*), reducing the magnitude of allelism testing of *mut5-1* considerably.

Fortuitously, only *rad 51*-bearing strains were readily available. The results of a complementation test of *mut5-1* and *rad51-1* with regard to γ-ray sensitivity are contained in Table 22 and Figure 28. Homozygous *mut5-1* and *rad51-1*, heterozygous and wild type diploids are included for comparison. There is no appreciable complementation observed in the *mut5-1/rad51-1* diploid.

TABLE 22

Complementation test of *mut5-1* and *rad51-1* with respect to γ-ray sensitivity

Dose (krad)	Survival (%) KF265 (*mut5/rad51*)		KF264 (*rad51/rad51*)		KF202 (*mut5/mut5*)	
0	100	(570)*	100	(570)*	100	(442)*
4	14.7	(839)	10.0	(571)	20.7	(917)
8	5.09	(290)	1.98	(113)	3.39	(150)
12	.719	(410)	.602	(343)	.842	(372)

Dose (krad)	Survival (%) KF266 (*rad51/* +)		KF204 (*mut5/* +)		KF205 (+ / +)	
0	100	(715)*	100	(612)*	100	(796)*
10	78.3	(560)	65.2	(399)	94.0	(748)
20	48.7	(348)	29.4	(125)	73.9	(588)
30	8.55	(611)	3.37	(206)	42.7	(340)
40	2.00	(143)	1.25	(767)	15.5	(123)

* Colony counts on which survival frequency based

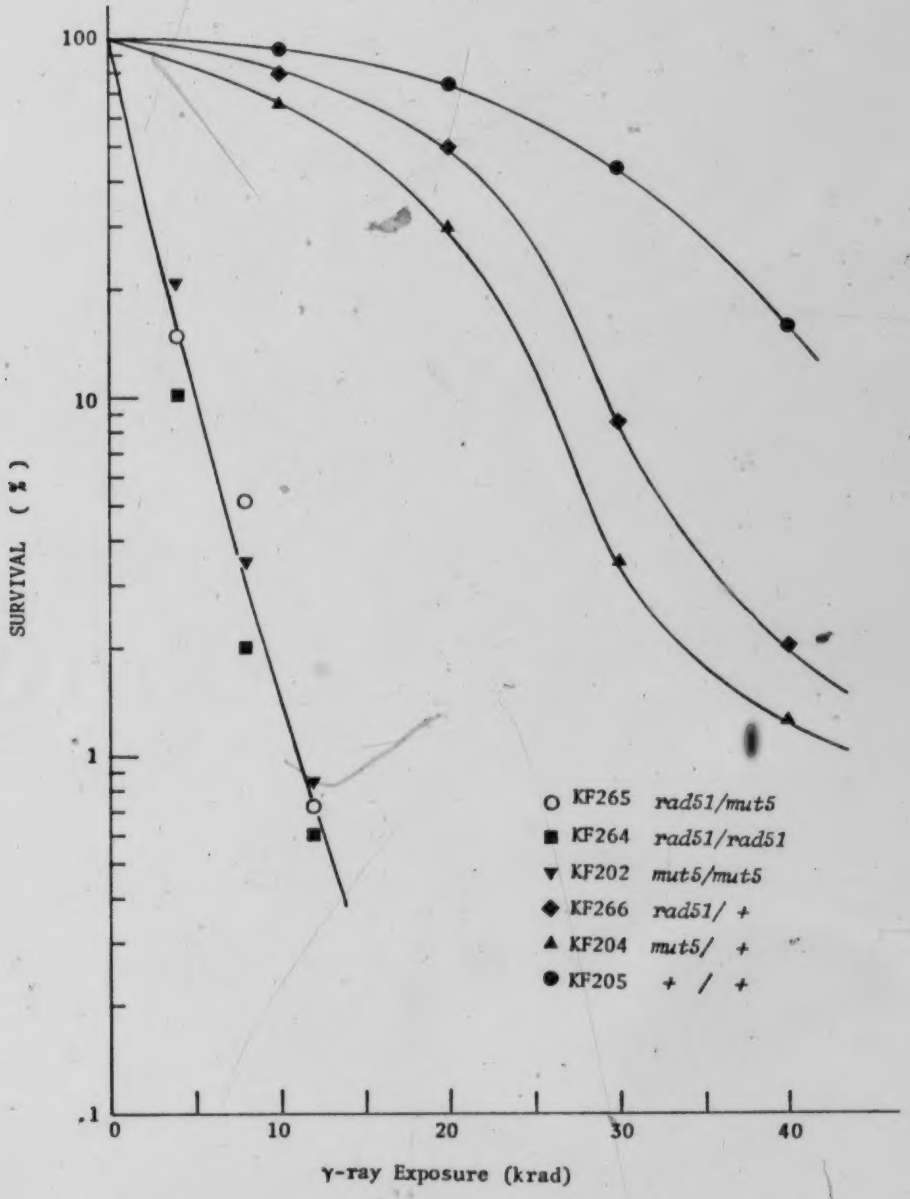

Figure 28 Complementation test of *mut5-1* and *rad51-1* with respect to γ-ray sensitivity

Survival curves of diploid strains carrying *mut5-1*, *rad51-1*, both or neither.

Lending appreciable weight to the view that *mut5-1* and *rad 51-1* are allelic was the subsequent observation that diploids carrying both do not sporulate at all well. The frequency of sporulation was estimated to be about 20%, most asci containing only 2 rather ill-defined spores. The viability of random spores obtained from 2 clones of KF263 were 5.6×10^{-4} and 1.7×10^{-4} viable spores/spore plated (Table 23). It must be noted that these are at best very crude estimates. The abnormal appearance of the spores made it difficult to identify them with any certainty in the post-glusulase digestion suspensions. None of 199 spore clones tested was γ-ray resistant.

Mapping mut 5 (rad 51)

Data obtained during the construction of strains carrying *mut5-1* and the markers to be used to check recombination confirmed the observation of S.-K. Quah (unpublished results) that *mut5-1* is linked to *his 1*. The other markers made it possible to place *mut 5* distal to *his 1* (Table 24A). The linkage of *rad 51* to *his 1* was shown in the analysis of the data of M. Mowat (unpublished results) which are presented with his permission in Table 24B. Subsequent analysis of crosses involving *trp 2* and *mut5-1* or *rad51-1* and various other markers, indicated that both *mut5-1* and *rad51-1* are very tightly linked to *trp 2* on the distal side (Table 24C and D).

The data of Game (1971) indicated that *rad 3* and *rad 4* are linked to *his 1*. The possibility that these loci were closely linked to *mut 5* (*rad 51*) was investigated. The results (Table 24E) indicate that while they are linked, *mut 5* (*rad 51*) and *rad 4* are not close. The detection of linkage of *rad 51* with *rad 4*, but not with *rad 3*, and the linkage of *rad 3* with *rad 4* (Table 24F; Game, 1971), places *rad 3* distal to *rad 4*.

TABLE 23

Viability of random spores from diploid strain KF263 (*mut5-1/rad51-1*)

	Clone 1	Clone 2
Estimated spore concentration in post-digestion suspension	4.2×10^5 spores/ml	3.5×10^5 spores/ml
Frequency of viable products	237/ml	61/ml
Estimated viability	5.6×10^{-4} viable spores/spore plated	1.7×10^{-4} viable spores/spore plated

TABLE 24

Mapping *mut 5* (*rad 51*): tetrad analysis data

Diploid	Pertinent genotype	Region	Tetrad type PD	NPD	T
A. KF178*	*his1 arg6* / + + *mut5*	*his1 - arg6*	40	0	11
		arg6 - mut5	31	1	19
		his1 - mut5	23	0	28
KF179*	*ura3 hom3* / + + *arg6 mut5*	*ura3 - hom3*	4	1	20
		hom3 - arg6	20	0	4
		arg6 - mut5	14	0	10
		hom3 - mut5	11	0	14
B. LA1	*his1* / + *rad51*	*his1 -rad51*	45	2	62
LA2	*his1* / + *rad51*	*his1 -rad51*	36	1	57

**mut 5*-bearing isolates were identified in these crosses on the basis of mutator activity and γ-ray sensitivity. No separation of the phenotypes was observed in 76 tetrads. In all further crosses *mut 5*-bearing strains were identified on the basis of their γ-ray sensitivities.

TABLE 24 (cont'd)

	Diploid	Pertinent genotype	Region	Tetrad type PD	NPD	T
C.	KF246	*hom3 + trp2 +* / *+ arg6 + mut5*	*hom3 - arg6*	63	0	17
			arg6 - trp2	47	0	31
			trp2 - mut5	75	0	3
			mut5 - arg6	44	0	35
	KF253	*hom3 + mut5* / *+ trp2 +*	*hom3 - trp2*	28	1	33
			trp2 - mut5	60	0	4
			hom3 - mut5	28	2	32
	KF254	*his1 trp2 +* / *+ + mut5*	*his1 - trp2*	43	0	45
			trp2 - mut5	82	0	7
			his1 - mut5	41	1	46
	KF255	*his1 trp2 +* / *+ + mut5*	*his1 - trp2*	23	0	15
			trp2 - mut5	34	0	4
			his1 - mut5	21	0	17
D.	KF247	*hom3 trp2 +* / *+ + rad51*	*hom3 - trp2*	36	3	33
			trp2 -rad51	72	0	1
			hom3 -rad51	36	3	34
	KF250	*his1 trp2 rad51* / *+ + +*	*his1 - trp2*	11	0	6
			trp2 -rad51	15	0	2
			his1 -rad51	9	0	8
E.	KF232	*his1 rad51 +* / *+ + rad4*	*his1 -rad51*	110	1	136
			rad51- rad4	61	18	190
			his1 - rad4	41	29	188
	KF234	*ura3 hom3 his1 mut5 +* / *+ + + + rad4*	*ura3 - hom3*	23	8	60
			hom3 - his1	81	0	8
			his1 - mut5	37	2	52
			mut5 - rad4	28	10	54
			his1 - rad4	18	18	55
	KF240	*his1 rad51 +* / *+ + rad3*	*his1 -rad51*	22	0	37
			rad51- rad3	9	6	47
			his1 - rad3	9	7	42

TABLE 24 (cont'd)

Diploid			Region	Tetrad type PD	NPD	T
F.	KF244	*hom3 arg6 rad4 +* / *+ + + rad3*	*hom3 - arg6*	43	0	10
			arg6 - rad4	8	9	36
			rad4 - rad3	38	0	15
			arg6 - rad3	7	12	34
	KF252	*ura3 his1 arg6 rad4 rad3* / *+ + + + + rad53*	*ura3 - his1*	5	5	31
			his1 - arg6	38	0	4
			arg6 - rad4	8	8	27
			rad4 - rad3	33	0	10
			arg6 - rad3	8	7	28
			rad53- ura3	8	4	30
			rad53- rad3	11	8	24
			rad53- his1	5	11	26
G.	KF248	*hom3 his1 trp2* / *+ + + rad54*	*hom3 - his1*	57	0	5
			his1 - trp2	36	1	25
			trp2 -rad54	12	14	37
			hom3 -rad54	12	10	42
	KF238	*ura3 hom3 his1 arg6 mut5* / *+ + + + + rad54*	*ura3 - hom3*	19	2	51
			hom3 - his1	67	0	6
			his1 - arg6	60	0	13
			arg6 - mut5	29	4	39
			mut5 -rad54	12	11	48
			his1 -rad54	14	11	47
			ura3 -rad54	10	9	52

Game and Mortimer (1976) somewhat tentatively suggested that *rad 51*, *rad 53* and *rad 54* are loosely linked. No linkage of *rad 54* with *mut 5* or any of the other markers was detected in this study (Table 24G). Crosses involving *rad 53* and *rad 54*, *rad 51-1* or *mut5-1* were made and finally abandoned , as a source of linkage data for *rad 53*, when it proved impossible to unambiguously identify *rad 53* spore clones by complementation testing. The one cross in which *rad 53* could be scored did not require this testing, and gave no indication of linkage of *rad 53* with *ura 3* or *rad 3* on the extremes of the marked region, or with *arg 6*, in the middle (Table 24F).

The Effect of *mut5-1* on Spontaneous Mitotic Recombination

The initial observation that *mut5-1* reduced the frequency of UV-induced intragenic recombination, but had no apparent effect on induced homozygosis, led to the testing of the unselected clones on the YD survival plates for homozygosis products, to confirm the *ade 2* results. Clones of homozygous *mut 5*, heterozygous and wild type strains (see Figure 29 for genotypes) were picked to YD medium, incubated for 24-48 hours and subsequently replica plated to omission media. After 3 or 4 days--prolonged incubation was necessary because many of the clones were very slow growing--the replicates were scored. The results are presented in Table 25.

The segregation of recessive markers in the wild type strain conformed to expectations (cf. Nakai and Mortimer, 1969): *arg 6* is distal to *hom 3* and is expected to be rendered homozygous more frequently: *ura 3* is relatively close to the centromere on the left arm and should be uncovered less often: the frequency of segregants increases with dose. One of the heterozygotes, somewhat unexpectedly, produced segregants in which a recessive marker on each side of the centromere was expressed. In

Figure 29

Configuration of pertinent markers on linkage group V* of strains used to check the segregation of recessive markers in unselected clones

KF205	*ura3 hom3* + +	
	+ + *arg6* +	
KF203	*ura3 hom3* + +	
	+ + *arg6 mut5*	
KF204	*ura3 hom3* + *mut5*	
	+ + *arg6* +	
KF202	*ura3 hom3* + *mut5*	
	+ + *arg6 mut5*	
KF223	*ura3* + *arg6 mut5*	
	+ *hom3* + *mut5*	

* Mortimer and Hawthorne, 1973

TABLE 25

Segregation of recessive auxotrophic markers in unselected clones of homozygous *mut5-1*, heterozygous and wild type strains

Strain	*MUT5* genotype	UV dose (J/m^2)	Number of clones checked	Number of auxotrophic segregants*				
				ura$^-$	*mth*$^-$	*arg*$^-$	*ura*$^-$*mth*$^-$	*ura*$^-$*arg*$^-$
KF205	+ / +	0	200					
		63	200		1	1		
		126	200	1	8	10		
KF203	*mut5*/ +	0	200					
		63	200		2	1	1	
		126	186	1	8	6	3	1
KF204	*mut5*/ +	0	200					
		63	200			1		
KF202	*mut5*/*mut5*	0	528			3	1	
		63	398	1		4	4	
		126	398	3	7	10	9	
KF223	*mut5*/*mut5*	0	426		3			2
		63	400	1	10			8
		126	400	3	11	6		15

* ura$^-$ indicates failure to grow on -ura medium (*ura3* uncovered)

arg$^-$ indicates failure to grow on -arg medium (*arg6* uncovered)

mth$^-$ indicates failure to grow on medium lacking methionine and threonine (*hom3* uncovered)

the *mut 5* homozygotes this tendency was enhanced. Not only were they produced at increasing frequency with increasing dose, some had in fact occurred spontaneously. That such segregants were produced spontaneously in strains carrying *mut 5* was of greater interest, their appearance suggesting increased frequency of multiple homozygosis events or of mitotic non-disjunction.

Confirmation that the results were real and reproducible was sought using strains KF256, KF257, KF258, KF259, KF260 and KF261. The linkage group V marker configurations were as follows:

KF256	*can1*	*ura3*	*hom3*	*his1*	*mut5*
and KF259	+	+	+	+	*mut5*
KF257	*can1*	*ura3*	*hom3*	*his1*	*mut5*
	+	+	+	+	+
KF260	*can1*	*ura3*	*hom3*	*his1*	+
	+	+	+	+	*mut5*
KF258	*can1*	*ura3*	*hom3*	*his1*	+
and KF261	+	+	+	+	+

The use of *can 1* precluded the use of *arg 6*. *CAN 1* encodes arginine permease (Grenson et al, 1966), mutants of which fail to take up the arginine analogue, canavanine, making the cells resistant to its killing effects. *can 1* is ordinarily only used in arginine-independent strains. *can 1 arg 6* strains can be maintained with ornithine supplementation (ornithine uptake is by another permease) but in my hands the growth tended to be very slow. Coupled with the slow growth of some of the unselected clones referred to earlier it made such a scheme impracticable. The very close linkage of *mut 5* to *trp 2* thwarted the ready preparation of a suitable set of strains carrying *trp 2*.

Zygotic clones were isolated for each diploid. After 2, 3 or 4 days growth the clones were picked, suspended and diluted in distilled water, and samples plated on can medium to select for canavanine resistant (can R) clones and on YD to establish the number of viable cells. Following 5 days incubation the number of colonies on each plate type was scored, and samples of can R clones picked to YD medium. After a further 3-4 days incubation, these were replica plated to can, -ura, -his and -mth media. The replicates were scored after 3 days. The results are presented in Table 26.

It is difficult to say much about the frequencies of can R clones obtained, beyond the fact that for homozygous *mut 5* strains they appear to be higher than those for the heterozygous and wild type strains.

Interesting comparisons, however, can be made concerning the nature of the segregants obtained. As with the initial experiment using unselected clones, the wild type strains again conform to expectation. *CAN 1*, being distal to *URA 3*, is subject to homozygosis more often, and this was observed for each of the samplings of 4-day old clones. The numbers of clones analyzed for the 2- and 3-day old clones were smaller and may have shown "jackpot" effects--the occurrence of a more or less rare event early in the zygotic clone growth resulting in inordinately high frequencies later when samples were plated. The low numbers of can R ura$^-$ mth$^-$ his$^-$ clones observed are consistent with such segregants being the result of 2 events, one proximal to *ura 3* on the left arm, the other proximal to *hom 3* on the right.

Among the can R isolates from the *mut 5* homozygotes the order of the frequenices of the 3 types is reversed. The class that would ordinarily be described as being the result of double events is in all 4 cases very large. Moreover, the number of can R ura$^-$ segregants exceeds the

TABLE 26

Segregation of recessive auxotrophic markers in selected canavanine-resistant clones

Strain	*MUT 5* genotype	Age of clone (days)	Frequency of can R clones ($\times 10^{-5}$)	Number of can R clones checked	Number of clones which were:			
					can R only	ura^-	can R and $ura^- mth^- his^-$	other
KF258	+ / +	2	4	8	2	2	4	
KF261	+ / +	3	6.83	69	13	51	5	
KF258	+ / +	4	1.95	400	310	85	5	
KF261	+ / +	4	11.6	292	198	92	2	
KF257	*mut5*/ +	2	59	78	24	2	52	
KF260	*mut5*/ +	3	7.3	85	22	17	46	
KF257	*mut5*/ +	4	1.61	298	126	49	122	1 $ura^- mth^-$
KF260	*mut5*/ +	4	3.68	209	94	16	99	
KF256	*mut5/mut5*	2	173	286	3	8	274	1 $mth^- his^-$
KF259	*mut5/mut5*	3	265	693	5	11	677	
KF256	*mut5/mut5*	4	44.8	198	5	8	185	
KF259	*mut5/mut5*	4	207	297	9	8	280	

number of can R-only clones. In classical terms this order of frequencies is not expected. The protocol used did not eliminate the possibility that the aberrant segregants for any one clone were the result of a very early event. That all 4 *mut 5* homozygous clones showed increased frequencies of unexpected segregants argues convincingly, but not conclusively, against such "jackpot" effects. Furthermore, the heterozygotes give relative frequencies that are between the wild type and *mut 5/mut 5* extremes.

Having established that the distorted segregation pattern of *mut 5*-bearing strains was real, an attempt was made to determine the nature of the mechanism causing it.

The two obvious possibilities are elevated frequencies of particular mitotic intergenic recombination events or mitotic non-disjunction. Sporulating the can R, multiply auxotrophic strains derived from the *mut 5* homozygotes and checking the viability of the meiotic products could not be done. Although *mut 5/mut 5* diploids do sporulate to some extent, tetrads with 4 viable products have never been isolated. Aneuploidy of presumptive (2n-1) strains should be readily identified by the inviability of 2 products from each tetrad. Inherently good spore viability is however essential, and homozygous *mut 5* diploids simply do not have it.

If the intermediate frequency of can R ura$^-$ mth$^-$ his$^-$ segregants seen in heterozygous *mut 5* strains is really a result of the incomplete dominance of *mut 5*, the expectation that their linkage group V constitution is the same as that for the *mut 5/mut 5* isolates of the same phenotype is not unreasonable. Figure 30 shows the predicted chromosome V configurations of such segregants from both heterozygotes for both hypotheses. It is readily seen that if either of these processes was involved, the segregants from KF257 will be phenotypically *mut 5*, and as

Figure 30

Chromosome configurations predicted for aberrant segregants occurring as a result of a double recombination event, or non-disjunction, in heterozygous *mut 5* diploids

Strain KF257

can1 ura3 hom3 his1 mut5
+ + + + +

double recombination event

can1 ura3 hom3 his1 mut5
can1 ura3 hom3 his1 mut5

non-disjunction

can1 ura3 hom3 his1 mut5

Strain KF260

can1 ura3 hom3 his1 +
+ + + + *mut5*

double recombination event

can1 ura3 hom3 his1 +
can1 ura3 hom3 his1 +

non-disjunction

can1 ura3 hom3 his1 +

TABLE 27

Viability of spores obtained from can R ura$^-$ mth$^-$ his$^-$ isolates of heterozygous *mat 5* diploids KF257 and KF260 and wild type diploids KF258 and KF261

A. KF257 - 2 day clone

Isolate	Number of complete tetrads dissected	Number of tetrads with: 0 viable spores	1	2	3	4
1	10	7	3			
2	10	8	2			
3	10	8	2			
4	10	8	1	1		
5	20	3	15	2		
6	10	3	6	1		
7	10	7	2	1		
8	10	8	2			
9	10	8	1	1		
10	10	5	5			
11	10	7	3			

TABLE 27 (cont'd)

B. KF260 - 3 day clone

Isolate	Number of complete tetrads dissected	Number of tetrads with: 0 viable spores	1	2	3	4
1	10	1	1	2	2	4
2	10			2	5	3
3	10	1	2	2	3	2
4	10			2	2	8
5	10				1	9
6	10				1	9
7	10				2	8
8	10				4	6
9	10				3	7
10	10			1	2	7
11	10			2	1	7
12	10		1	1	2	6
13	10			1	3	6
14	10		1	2	2	5
15	11				6	5

TABLE 27 (cont'd)

C. KF258 and KF261 - 2, 3 and 4 day clones

Isolate*	Number of complete tetrads dissected	Number of tetrads with: 0 viable spores	1	2	3	4
1	10		1		2	7
2	10	1		5	3	1
3	10			2	3	5
4	10			1	4	5
5	10			2	4	4
6	10			4	4	2
7	10				2	8
8	10			1	5	4
9	10			1	2	7
10	10			3	3	4
11	10				1	9
12	10				5	5

* Isolates 1 and 2 KF258 (2 day)
Isolates 3-6 KF258 (4 day)
Isolates 7-10 KF261 (3 day)
Isolates 11 and 12 KF261 (4 day)

such are not expected to produce many viable products on sporulation. The KF260 segregants are expected to be phenotypically wild type and as such should give good viability of meiotic products. If non-disjunction were the cause, good viability would mean 2 viable products only per tetrad.

Dissection of can R ura$^-$ mth$^-$ his$^-$ isolates from KF257 confirmed the prediction that the viability of meiotic products would be poor (Table 27A). No tetrad gave 3 or 4 viable products, and only 6 of 120 asci produced two viable spores. The overall viability was just over 11%.

The data for strain KF260 and control wild type isolates are shown in Table 27B and C respectively. The viability of the meiotic products of the aberrant segregants derived from KF260 is as good as that for the wild type isolates. That tetrads containing 3 or 4 viable products are obtained from every isolate indicates that not every cell, if any, of these isolates was a hypodiploid.

mut 5 and Meiotic Recombination

This analysis was carried out in an effort to ascertain whether any *mut 5* effect on meiotic recombination was evident in the relatively infrequent viable products of sporulation. As with the *mut5-1/rad 51* diploids it was very difficult to obtain precise frequencies of viable spores for *mut5-1* homozygotes. The estimated range was 5×10^{-4} - 5×10^{-3} viable spores/spore plated. This viability estimate for *mut5-1/mut5-1* random meiotic products is not consistent with that reported earlier for spores dissected from complete tetrads from diploids which were predicted to be homozygous or hemizygous *mut5-1*. The latter may however be a very select subpopulation. Viability of random spores from heterozygotes and wild types was 50-80%.

Table 28 shows the frequencies of selected histidine prototrophs obtained from 3 different *mut5-1* homozygotes, 2 heterozygotes and 2 wild type strains, all of which were heteroallelic at *his 1*. The poor sporulation and viability of meiotic products of *mut5-1* homozygotes is reflected in the low numbers of histidine prototrophs observed for strains KF223, KF224 and KF225. The frequencies of prototrophs per viable spore, however, are not unlike those obtained for the heterozygous and wild type strains.

Estimates of intergenic recombination frequencies in the *hom 3-arg 6* interval were obtained by streaking unselected clones on YD medium, incubating them overnight and then replica plating them on -mth and -arg media to score for the presence of *hom 3* and *arg 6* respectively. The results (Table 29) again fail to show any effect of *mut5-1*.

Another Approach to Meiotic Recombination

Synchronous sporulation of yeast is induced by transferring heterozygous mating-type diploids to sporulation medium consisting of 1% potassium acetate in distilled water (Roth and Halvorson, 1969). This initiates the differentiation process leading to meiosis. Sherman and Roman (1963) showed that such diploids, when removed from sporulation medium and plated on nutrient medium, were capable of return to vegetative growth, and, further, that the frequency of intragenic recombinants among cells which had reverted increased with the length of exposure to sporulation medium. It is now generally accepted that the recombination observed on return to vegetative growth is in essence meiotic recombination (Roth and Fogel, 1971; Silva-Lopez et al, 1975; Esposito and Esposito, 1974; Hopper and Hall, 1975). On this basis it is expected that diploid strains defective in meiotic recombination processes might also prove deficient in this "parameiotic" recombination. The results of an experi-

TABLE 28

Intragenic recombination in random meiotic products of homozygous *mut5-1*, heterozygous *mut5-1* and wild type diploids

Genotype	Strain	Frequency of histidine prototrophs (/10^3 viable spores)
$\frac{his1\text{-}315\ mut5\text{-}1}{his1\text{-}1\ mut5\text{-}1}$	KF223	1.8 (19)*
	KF224	1.5 (18)
	KF225	2.4 (14)
$\frac{his1\text{-}315\ mut5\text{-}1}{his1\text{-}1\ +}$	KF203	2.22 (1906)
$\frac{his1\text{-}315\ +}{his1\text{-}1\ mut5\text{-}1}$	KF204	2.47 (2861)
$\frac{his1\text{-}315\ +}{his1\text{-}1\ +}$	KF205	3.39 (1388)
	KF229	1.87 (3361)

* Colony count on which frequency based

TABLE 29

Intergenic recombination in unselected random meiotic products of homozygous *mut5-1*, heterozygous *mut5-1* and wild type diploids

Genotype	Strain	Number of unselected clones scored	Number of recombinants		Recombination frequency (%)
			hom3 arg6	+ +	
$\frac{+\ arg6\ mut5\text{-}1}{hom3\ +\ mut5\text{-}1}$	KF223	208	18	16	16.3
	KF224	208	14	9	11.1
	KF225	196	11	16	13.6
$\frac{+\ arg6\ mut5\text{-}1}{hom3\ +\ +}$	KF203	208	12	14	12.5
$\frac{+\ arg6\ +}{hom3\ +\ mut5\text{-}1}$	KF204	196	7	15	11.2
$\frac{+\ arg6\ +}{hom3\ +\ +}$	KF205	166	14	15	17.5
	KF229	468	28	32	12.8

ment to assess the effect of *mut5-1* in such a system are presented in Table 30 and Figure 31.

The parameter "relative viability" provides a rather crude measure of the ability of acetate-exposed cells to continue vegetative growth. Nevertheless, two points can be made: for all strains there appeared to be a slight increase in the number of plating units following exposure to sporulation medium, and a marked decline in colony-forming ability was observed at 44 hours.

At 3 hours the frequencies of prototrophs induced by exposure to sporulation medium in the 2 clones of KF202 and KF205 were essentially the same. In the 8h and succeeding samples however, the wild type frequency was clearly greater. The maximum frequencies obtained for the *mut5-1* homozygotes (at 24h) were an order of magnitude less than that of the wild type control (at 44h). Both homozygous *mut5-1* clones exhibited a very large decrease in prototroph frequency at 44h.

mut5-1, Mating-type and γ-ray Inactivation

The epistatic interaction on X-irradiation of *rad 51* and *rad 52* (Game, cited by Haynes, 1975) and the failure of *rad 52/rad 52* diploids to show the "*a*/α effect" (Ho and Mortimer, 1973), leads to the prediction that *mut 5/mut 5; a*/α strains may also fail to show any decrease in γ-ray sensitivity compared to *mut 5/mut 5; a/a* strains. The results of an experiment to test this prediction are presented in Table 31 and Figure 32. The increased resistance to γ-ray inactivation of the wild type isolate KF205-R17 (*a*/α) relative to KF205-R28 (*a/a*) is very clear, as is the failure of *a*/α to increase the radioresistance of the *mut 5* homozygote KF202-R17 relative to KF202-R28 (*a/a*).

TABLE 30

The effect of *mut5-1* on parameiotic recombination

A. KF202 - clone 1 (*his1-315 mut5-1/his1-1 mut5-1*)

Time in sporulation medium (h)	Relative viability[1]	Frequency of asci (%)	Frequency of histidine prototrophs (/10^4 cells plated)[2]
0	1 (275)[3]		(0)
3	1.3 (362)		(0)
8	1.1 (302)		.094 (54)
13	1.0 (280)		1.02 (589)
16	1.0 (285)		1.93 (1107)
19	.84 (231)	<.1 (0:1059)[4]	3.00 (1728)
24	1.2 (316)	2.4 (27:1109)	3.30 (380)
44	.58 (160)	10.4 (71:680)	.45 (52)

[1] To = 1

[2] As determined by hemocytometer count

[3] Colony counts on which frequencies based

[4] (Number of asci observed:number of cells scored)

TABLE 30 (cont'd)

B. KF202 - clone 2 (*his1-315 mut5-1/his1-1 mut5-1*)

Time in sporulation medium (h)	Relative viability	Frequency of asci (%)	Frequency of histidine prototrophs (/10^4 cells plated)
0	1 (187)		
3	1.0 (196)		
8	.89 (166)		.031 (11)
13	1.1 (198)		.709 (248)
16	.97 (182)		1.35 (474)
19	1.0 (192)		1.79 (627)
24	.88 (164)		2.14 (150)
44	.51 (96)	6.9 (42:606)	.33 (23)

C. KF203 (*his1-315 mut5-1/his1-1 +*)

Time in sporulation medium (h)	Relative viability	Frequency of asci (%)	Frequency of histidine prototrophs (/10^4 cells plated)
0	1 (166)		.003 (1)
3	1.3 (220)		.006 (2)
8	1.1 (190)		.009 (3)
13	1.4 (237)		.23 (73)
16	1.3 (211)		1.30 (416)
19	1.0 (168)	<.1 (0:1021)	3.65 (1163)
24	1.7 (280)	1.0 (3:301)	11.1 (709)
44	.95 (158)	30.7 (189:616)	20.4 (692)

TABLE 30 (cont'd)

D. KF204 (*his1-315 + /his1-1 mut5-1*)

Time in sporulation medium (h)	Relative viability	Frequency of asci (%)	Frequency of histidine prototrophs (/10^4 cells plated)
0	1 (76)		
3	1.1 (80)		
8	1.3 (97)		
13	1.1 (84)		.10 (13)
16	1.2 (94)		2.33 (296)
19	1.3 (100)	<.2 (0:590)	7.41 (941)
24	1.1 (82)	12.2 (41:336)	19.1 (484)
44	.83 (63)	48.0 (200:417)	27.2 (346)

E. KF205 (*his1-315 + /his1-1 + *)

Time in sporulation medium (h)	Relative viability	Frequency of asci (%)	Frequency of histidine prototrophs (/10^4 cells plated)
0	1 (431)		.147 (113)
3	1.12 (482)		.152 (117)
8	1.05 (452)	<.1 (0:1061)	.222 (171)
13	1.05 (452)	.4 (4:1006)	2.71 (2090)
16	1.15 (497)	1.6 (16:1016)	6.44 (1239)
19	1.02 (439)	1.9 (12:639)	9.10 (875)
24	1.54 (664)	11.1 (111:1004)	24.7 (950)
44	.53 (228)	51.0 (274:537)	39.5 (380)

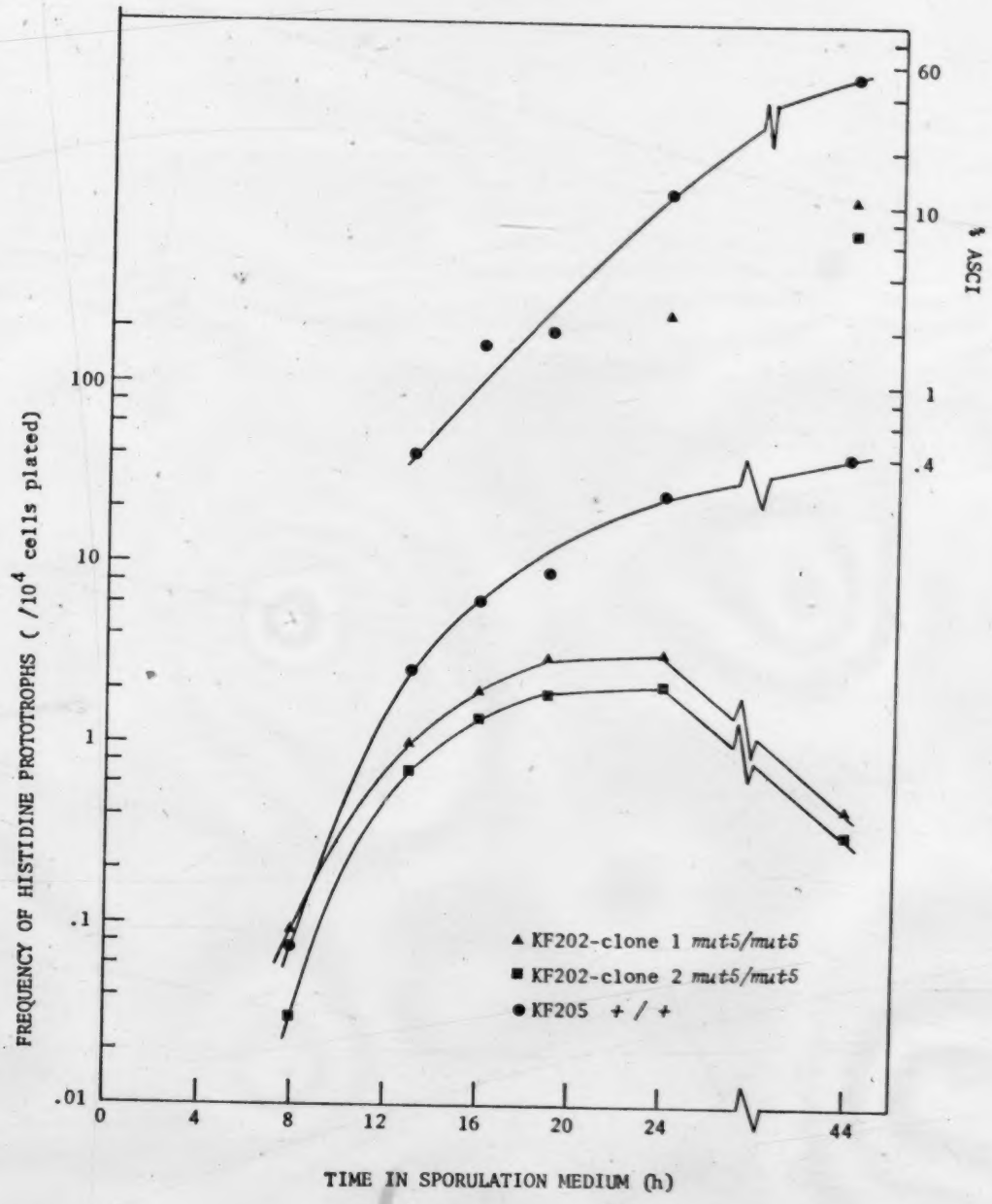

Figure 31 Intragenic recombination at *his 1* following exposure to sporulation medium of homozygous *mut5-1* and wild type diploids

TABLE 31

The effect of mating-type constitution on γ-ray inactivation of *mut5-1* homozygous and wild type diploids

KF202-R15 (*a/α;mut5/mut5*)		KF202-R25 (*a/a;mut5/mut5*)		KF205-R17 (*a/α; + / +*)		KF205-R28 (*a/a; + / +*)	
Dose (krad)	Survival (%)	Dose (krad)	Survival (%)	Dose (krad)	Survival (%)	Dose (krad)	Survival (%)
0	100 (336)*	0	100 (421)*	0	100 (626)*	0	100 (487)*
2	56.8 (191)	2	61.5 (259)	10	85.5 (535)	4	93.6 (456)
4	28.6 (96)	4	30.6 (129)	20	69.5 (435)	8	73.7 (359)
8	5.90 (991)	8	3.92 (1652)	30	53.0 (332)	12	55.0 (268)
12	1.33 (560)	12	.531 (1118)	40	37.1 (232)	16	42.9 (209)
16	.345 (1160)	16	.208 (874)	50	25.9 (162)	20	24.3 (1185)
20	.148 (496)	20	.109 (458)			30	11.0 (534)
30	.013 (44)	30	.004 (17)			40	6.12 (298)

* Colony counts on which survivor frequency based

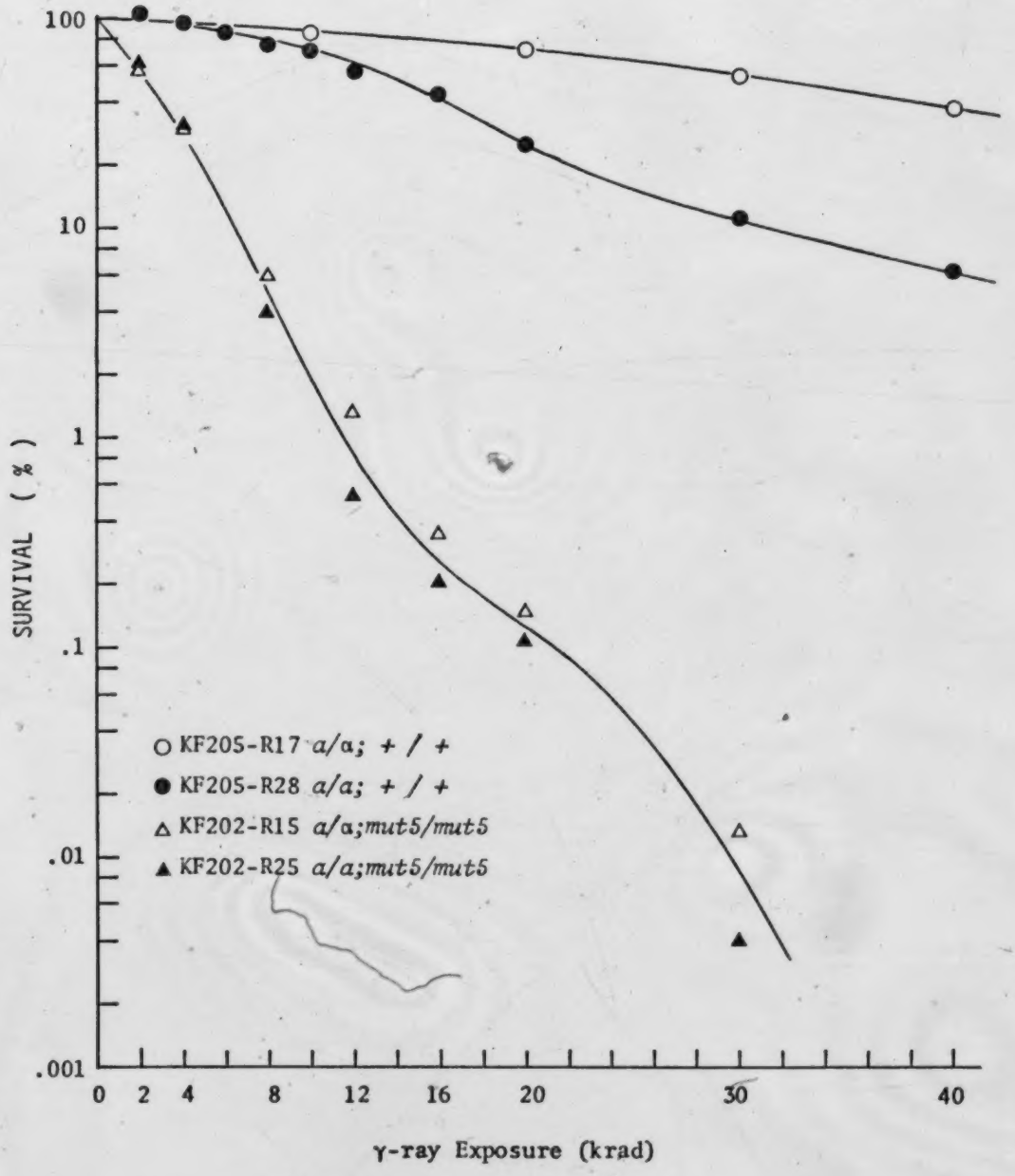

Figure 32 Survival after γ-irradiation of *mut5/mut5* and wild type diploids heterozygous or homozygous for mating-type

Mating Type and Mutators

The observation of S.-K. Quah (unpublished results) that the expression of mutator activity, as measured in both the Lassie test and the 1000-compartment fluctuation test (von Borstel et al, 1971), was reduced in homozygous *mut 4* or *mut 3* diploids, relative to the haploid parents, prompted speculation that spontaneous mutability might be subject to an "*a*/α effect" analogous to that described for X-ray inactivation of diploids (Mortimer, 1958; Laskowski, 1962). The results of an investigation to attempt to clarify this situation follows.

Homozygous mutator, heterozygous and wild type diploids which were $\frac{a\ cry1}{\alpha\ +}$, $\frac{a\ cry1}{\alpha\ cry1}$, and $\frac{a\ cry1}{a\ cry1}$ were prepared as described in "Materials and Methods", and subjected to Lassie testing. The data are presented in Tables 32-39.

No effect of homozygosity of mating-type is seen in the *mut 1* data (Table 32). There are clearly no major differences between the cryptopleurine-resistant (cry R) isolates and the $\frac{a\ cry1}{\alpha\ +}$ strains from which they were derived.

The data for the *mut 2* set (Table 33) are incomplete. When the *a cry1*/α + isolates of one of the heterozygotes and the wild type strain were spread on *cry* medium to select cry R recombinants, lawns rather than the expected few discrete colonies grew up. It was inferred that the clone of the common wild type haploid used in the mating was derived from a cell that had mutated to cryptopleurine resistance. When the results from the other 2 strains were obtained, there was no point to repeating the experiment. The Lassie test results for the homozygous mutator and the second heterozygote show a general increase in all the cry R clones that appears to be attributable to *cry 1*, and not mating-type, homozygosity.

TABLE 32

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut1-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF186 (*mut1-1/mut1-1*)			KF187 (*mut1-1/ +*)			KF188 (*+ /mut1-1*)			KF189 (*+ / +*)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
1248	1972		43	45		29		41	18		48
1303	1121		45	74		36		53	24		49
1318	1149		47	47		52	35		25		34
1638	1254		47		44	52		75	25	34	
1743	1037		52		60	55		62	27		33
1889		1423	54	48		56	39		28	33	
1915		2120	57		44	60		61	29		
1953		2329	62		59	63		44	32	35	
1982			62		73	72		47	35		56
2198		1725	81	87		78		102	42		49

TABLE 33

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut2-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:					
KF190 (*mut2-1/mut2-1*)			KF191 (*mut2-1/ + *)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
156		392	4		
178		682	5		
182		744	6		69
183			7		
185		817	10	44	
203		408	10		55
204		360	10		
204	434		11		63
210		496	11		
226		649	12		52

The increase in mutator activity associated with *cry 1* homozygosity is seen again in the *mut 3* data (Table 34). However, a further marked increase beyond the *a cry1/α cry1* scores, was observed for the 4 *mut 3 a cry1/mut 3 a cry1* isolates. This appears to be related to mating-type homozygosity.

Each of the isolates homozygous for *mut 4* and *a* mating-type produced more revertants during limited growth than the *a cry1/α +* strain from which it was derived (Table 35). With only one exception *a cry1/a cry1* strains exhibited more spontaneous mutation than any of the *a cry1/α cry1* isolates. The exceptional isolate did however score higher than an *a cry1/α cry1* sister clone.

Comparison of the Lassie results of the *a cry1/α +* and *a cry1/α cry1* isolates of strains KF203, KF204 and KF205 (Table 36), shows again the mutation increase associated with *cry 1* homozygosity. Whether all of the increase that is seen in the *mut 5* heterozygotes (KF203 and KF204) can be also attributed to this is unclear. The data for the *mut 5* homozygous strains show no effect of mating-type constitution.

The results for *mut 6* and *mut 9* strains (Tables 37 and 38 respectively), while they do show the *cry 1* homozygosity effect, do not reveal any mating-type influence of these mutator mutants on spontaneous mutability.

Of the mutators used in this study *mut 10* is the weakest as far as Lassie tests are concerned. Haploid strains carrying *mut 10* routinely gave Lassie scores around 100, but were nevertheless identifiable among the meiotic products of *mut 10* heterozygotes. The results presented for *mut 10* homozygotes in Table 39 are therefore somewhat unexpected. In the *a cry1/α +* isolates the mutator phenotype is very clearly expressed. The *a cry1/a cry1* clones, on the other hand, exhibit a general decline to a level not unlike that of *mut 10* haploids. No changes in revertant

TABLE 34

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut3-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF194 (*mut3-1/mut3-1*)			KF195 (*mut3-1/ +*)			KF196 (*+ /mut3-1*)			KF197 (*+ / +*)		
a cry1 / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*
48			4	38		12		38	7	20	
49			5			15	52		7		28
50	120		9		30	17		44	8		18
53		398	10			18		58	8		
53		440	11		24	20		43	10	26	
56			12			21		54	10	50	
57	127		13			21		57	11	34	
61		299	14		23	21		54	11	42	
69	126		15			22	82		11	34	
94		325	23		48	28		41	11		61

TABLE 35 The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut4-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF198 (*mut4-1/mut4-1*)			KF199 (*mut4-1/ + *)			KF200 (+ /*mut4-1*)			KF201 (+ / +)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
48	78		21	53		24	27		28	52	40
65		174	27	38		26	36	41	32		63
66	78	120	35		52	30		50	32		
69		186	40	37	48	32		41	33		39
78		191	41		34	35		37	33	64	44
81	63		41		44	35	44	27	34	26	48
82		188	45		17	38	34		38		64
87	97		48	52		43		39	39	69	33
87	64		57	37		44		49	41		35
98	89		60			81	53	43	56	55	
100		180									
102		206									
104	63										
107											
111		170									
116	72	303									
152	92										
153		181									
189	161										
217	9	355									

TABLE 36

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut5-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of KF202 (*mut5-1/mut5-1*)

a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
188		290	253			296		270	378	276	
210	291		254	231		304	275		383	279	
216		237	260	292		306	447		388	193	
220	255		265	370		343		218	388	223	
221	421	372	268	309		345		207	400	257	
230	229		273		282	346	343		412		295
231			279	257		355		272	422		143
232	227		280	530		356		304	446	287	
234		419	283	224		363	272		465	238	
247	240		294			368	300		483		268
248	226		294	263		369	262		558		333
251	286		295	251		377	268				

TABLE 36 (cont'd)

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut5-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:								
KF203 (*mut5-1*/ +)			KF204 (+ /*mut5-1*)			KF205 (+ / +)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
20		152	14			7		38
25		170	16		117	13		36
30		105	17		160	14		38
32	99		19	82		15		32
35		125	20		139	15	54	
36		160	21		117	16		
37			21			21		27
40		160	22		140	23		38
42		153	22	98		24		
49		168	24			32		31

TABLE 37

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut6-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF206 (*mut6-1/mut6-1*)			KF207 (*mut6-1/ +*)			KF208 (*+ /mut6-1*)			KF209 (*+ / +*)		
a cry1 / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α *+*	*a cry1* / α *cry1*	*a cry1* / *a cry1*
77		167	20		62	12		72	16		
95		181	30		29	21		68	19		37
106		182	33	95		23	141		24		
113			35	35		25		96	24		34
125		233	37		64	27		94	25		
129	168		37	73		27		65	28		30
136	259		40		34	30	54		31	29	
143		171	42	52		31			32		30
186			43		83	33	95		34	27	
189			47		40	62		99	62		

TABLE 38

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut9-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF210 (*mut9-1/mut9-1*)			KF211 (*mut9-1/ +*)			KF212 (*+ /mut9-1*)			KF213 (*+ / +*)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
157			14	26		16			10	33	
161		158	20	31		18		78	17	21	
165		218	21			19		76	22		32
168	91		21		33	20		70	22	28	
176	214		22		40	21		72	27		39
180		244	23			25			27		34
209		218	23			26		86	27		75
221		235	23		40	27		67	28	45	
226		202	24	33		30	37		32		40
254			31		28	62			33		72
258			37								
263											

TABLE 39

The effect of mating-type genotype on spontaneous mutability in diploids carrying *mut10-1*

The number of lysine-independent revertants which arose during lysine limited growth of derivatives of:												
KF214 (*mut10-1/mut10-1*)			KF215 (*mut10-1/ +*)			KF216 (*+ /mut10-1*)			KF197 (*+ / +*)		
a cry1 / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*	*a cry1* / α +	*a cry1* / α *cry1*	*a cry1* / *a cry1*
161			45		59	16		58	7	20	
179			50		28	16		54	7		28
196		117	51	57		17		68	8		18
199		115	54	69		19		75	8		
200		67	55			20		76	10	26	
214		80	59		74	22		79	10	50	
219		126	62	67		23		79	11	34	
222		62	64		67	28	81		11	42	
228		113	70		51	35	76		11	34	
248		87	98			47		76	11		61

frequencies attributable to *mut 10* is seen in the heterozygotes. The *cry1/cry1* effect is seen in the wild type and one of the heterozygotes.

Summary of Results

Diploid strains carrying *mut1-1*, *mut2-1*, *mut6-1* or *mut9-1* all failed to exhibit any effect on UV-induced mutation or UV-induced intra- or intergenic recombination, and showed no change in spontaneous mutation frequencies as a consequence of altered mating-type constitution.

No influence of *mut3-1* or *mut4-1* on UV-induced mutation or recombination was detected. Homozygous *mut 3* or *mut 4* strains showed reduced mutator activity when they were heterozygous at the mating-type locus. Increased Lassie test scores were recorded when mating-type was rendered homozygous.

The response to changes in mating-type genotype of *mut10-1* homozygous diploids was unexpected. Homozygous *mut 10* a/α strains exhibited very clearly the mutator phenotype. Isolates of these strains which were homozygous *a* gave Lassie scores lower than those of their a/α progenitors.

mut5-1 homozygous, heteroallelic *his 1* diploids were shown to be deficient in the production of histidine prototrophs following UV-irradiation. Heterozygous *mut 5* strains appear to be intermediate. That induced mutation can account for most of the prototrophs seen in *mut 5/mut 5* diploids was shown using homozygous *his1-1* or *his1-315* strains. Essentially normal UV-induced reversion of *lys1-1* was seen in *mut 5*-bearing diploids.

While no effect of *mut 5* on induced homozygosis of *ade 2* was observed, unselected clones of heterozygous and homozygous *mut 5* strains indicated that spontaneous segregants were occurring in which two recessive markers, one on each arm of linkage group V, were being uncovered. This observa-

tion was confirmed using selected can R clones from *can1 ura3 hom3 his1/+ + + +* diploids. A check of the viability of apparent non-disjunction segregants from heterozygous *mut 5* strains failed to confirm non-disjunction as the cause.

Allelism of *mut5-1* with *rad51-1* was apparent in complementation and mapping studies. Linkage of *mut 5* (*rad 51*) to *trp 2* and *rad 4* was found. No linkage of *rad 53* or *rad 54* to other markers on the right arm of linkage group V was detected.

Intra- and intergenic recombinant frequencies in the viable products of meiosis in *mut 5/mut 5* strains appear to be normal. An investigation of sporulation parameters using parameiosis (return of cells to nutrient medium after exposure to sporulation medium) showed a failure of *mut 5* homozygotes to achieve wild type frequencies of intragenic recombination or ascus production. A decline in prototroph frequencies for *mut 5/mut 5* strains was seen at 44h, when the frequency in the wild type was still increasing.

No interaction of *mut 5* and mating-type was detected when Lassie tests were run on *a*/α and *a/a* isolates of diploids carrying *mut5-1*. The "*a*/α effect" on ionizing-radiation inactivation was not seen for homozygous *mut 5* diploids.

DISCUSSION

The characterization of yeast mutator mutants with regard to UV-induced mutation, UV-induced intra- and intergenic recombination and the effect of mating-type homozygosity on the expression of the mutator phenotype was carried out in an attempt to clarify the relationship of these mutants with the DNA repair systems.

In the case of *mut 1* and *mut 6*, neither of which is sensitive to UV-light, γ-irradiation or methylmethanesulphonate, an effect on any one of these parameters would have provided the first evidence (beyond the fact that they are mutators) that they might indeed be associated, however remotely, with the repair systems of yeast as they are currently understood. No clearly defined effect on these parameters was seen. The absence of any effect cannot in itself support the conclusion that these loci do not interact with the DNA repair systems.

The mutants that have been assigned to pathways were, with the exception of *rev 1*, *rev 2* and *rev 3*, originally isolated on the basis of sensitivity to UV-light or ionizing radiation. It is most unlikely that the lesions created by these agents are representative of all possible lesions. The functions encoded by *MUT 1* and/or *MUT 6* may be involved with the non-mutagenic processing of other specific lesions, which occur spontaneously, and which, if not resolved properly, may result in mutation. The dominance exhibited by *MUT 6* in some genetic backgrounds (Hastings et al, 1976) could reflect the extent to which such lesions occur in particular strains and the capacity of the heterozygote to deal with them.

An indication that *MUT 1* mutations may interact with a repair system in yeast comes from the observation of S.-K. Quah, R. C. von Borstel

and P. J. Hastings, unpublished results, that some antimutator mutants, which all but eliminate the mutator phenotype of *mut 1* in double mutants, are UV-sensitive.

Another possibility which is currently being investigated, (C. K. Tan and P. J. Hastings, personal communication), is that mutation of *MUT 1* or *MUT 6* may result in nucleotide pool imbalances, mutation rate increases occurring as a result of misincorporation and/or the subsequent misrepair of the lesions. Excessive incorporation of dUTP into DNA in mutants of *E. coli* lacking dUTPase, removal of the uracil by uracil-N-glycosidase and subsequent repair of the apyrimidinic sites, has recently been demonstrated by Tye et al, 1977. Such strains exhibit increased recombination and spontaneous mutation (cf. *pol A*$^-$ strains discussed earlier). Clearly any mutation which increased the extent of misincorporation at replication could result in the mutator phenotype.

Much of what has just been said about *mut 1* and *mut 6*, could easily apply to *mut 2*. While *mut 2* strains do exhibit MMS-sensitivity, the absence of UV- or X-ray-sensitivity, or any effect on UV-induced mutation or recombination suggests that it may belong in a pathway yet to be identified. In a recent study, (Prakash and Prakash, 1977) mutants isolated on the basis of their MMS-sensitivity and which complemented the existing *rad* mutants were shown to belong in 22 new complementation groups. Mutants in 5 of these complementation groups exhibit no sensitivity to UV- or X-irradiation. Until further characterization of mutants in these 5 groups is carried out one can only speculate as to their normal function. The existence of another repair pathway in which *MUT 2* has a part is not, however, an unreasonable conjecture.

The sensitivity of *mut9-1*-bearing strains to UV- and γ-irradiation

and to MMS made *MUT 9* a prime candidate for inclusion in the *RAD 18* or the *RAD 51* system. Here again, however, no effect was observed on the secondary phenotypes examined. On the hypothesis that the mutator phenotype is the result of redirection of spontaneous lesions from a blocked non-mutagenic repair system to a functional but mutagenic process, one would conclude that the spontaneous defect normally handled by *MUT 9*, whose involvement in radiation repair is minor, is not subject to repair by any of the defined systems. That additional systems may exist was indicated by the observations of Prakash (1974) that nitrous acid and nitrosoimidazolidone are mutagenic in otherwise immutable *rad 6* and *rad 9* strains, and of Brychcy (1974) that *rad6-1*-bearing strains exhibit the mutator phenotype.

A clear indication that mutator loci may be involved with DNA repair processes in yeast is seen in the interactions of *mut3-1* and *mut4-1* with mating-type. The failure of *a*/α strains homozygous for *mut 3* or *mut 4* to express the mutator phenotype, and the restoration of this phenotype in *a*/*a* diploids indicates that an interaction exists. The simplest and most compelling explanation, based on the observations of Mortimer (1958) and Laskowski (1962) is that spontaneous lesions, which result in mutation in haploids, are processed, preferentially and non-mutagenically, by an *a*/α-dependent repair system in heterozygous mating-type diploids. This system, it appears, is not used to any great extent or is not available in haploids or homozygous mating-type diploids. The latter is unlikely to be the case. The data of Game and Cox (1973) indicate an interaction of *rad 51* with other repair gene mutations in double mutant haploids exposed to UV-light. This implies that *RAD 51* function is available in haploids. That *RAD 51* is a component of the

a/α-dependent pathway is indicated by the failure of heterozygous mating-type to increase the radioresistance of *mut 5/mut 5* strains relative to *a/a;mut 5/mut 5* diploids (see Figure 32).

The absence of any effect of *mut3-1* or *mut4-1* on UV-induced recombination suggests that they do not result in channelling of appreciable numbers of UV-induced lesions to the *a*/α-dependent system, whose involvement with UV-induced recombination was indicated by Friis and Roman (1968). This is consistent with the observation that haploid strains carrying *mut3-1* or *mut4-1* are only weakly sensitive to UV-light.

The possibility cannot yet be ruled out that the absence or defectiveness of an enzymatic function in *mut 3* or *mut 4* mutants causes the production of DNA lesions which are subject to mutagenic repair in the absence of the *a*/α dependent process.

The few data that were obtained on *mut10-1*-bearing diploids indicate that this locus too interacts with the *a*/α repair process, but in a totally unexpected manner. Spontaneous mutability is decreased in *a/a; mut 10/mut 10* diploids relative to *a/α;mut 10/mut 10* strains. Assuming that mutation of *MUT 10* does not of itself increase the number of lesions, but rather, that *MUT 10* is involved in the processing of spontaneously-occurring lesions, it is possible that in *a/α mut 10/mut 10* strains these lesions, which give rise to some mutation in haploids and in *a/a* diploids, have an increased probability of being resolved mutagenically. If *MUT 10* is a component of the *a*/α-dependent system [a not unlikely supposition in view of the γ-ray sensitivity of *mut 10* strains (Hastings et al, 1976) and the poor sporulation exhibited by *mut 10* homozygous diploids (S.-K. Quah, personal communication)], then the preferential handling of lesions by this pathway in *a*/α strains, as indicated by the *mut 3* and *mut 4* results, would effectively increase the number of lesions prone to mutagenic re-

pair. How then might *mut 10* homozygosis result in increased spontaneous mutability in *a/α* diploids?

Two possibilities are suggested: a defective *MUT 10* gene product might act to resolve the lesion mutagenically, or, if the *MUT 10* gene product is absent, the secondary lesion may be redirected into a mutagenic process. No choice between these is possible on the basis of the data that has been collected.

If *mut 10*-bearing strains exhibit the mutator phenotype as a result of the production of lesions by a defective *MUT 10* gene product or by virtue of its absence, then it must be argued that these lesions are resolved preferentially and mutagenically by the *a/α* repair process. This would mean that the nature of the processing carried out by the *a/α* dependent process, mutagenic (as for *mut 10*) or non-mutagenic (as for *mut 3* and *mut 4*), was dependent on the lesion that it was called upon to handle. I do not think that we are as yet in a position to discount categorically this possibility.

Strains carrying *mut5-1* exhibit, in addition to the mutator phenotype, sensitivity to UV-light, X-rays and MMS (Hastings et al, 1976). Homozygous *mut5-1* diploids do not sporulate particularly well and show poor viability of the few meiotic products that are produced. This description applies as well to mutants of most of the *RAD 50* series genes (Game and Mortimer, 1974). It was not unduly surprising to find that *mut5-1* homozygous diploids are apparently totally devoid of any UV-induced intragenic recombination. Mutation of *RAD 51* or *RAD 52* (Saeki et al, 1974; Resnick, 1975) had also been shown to result in reduced frequencies of induced intragenic recombinants.

The failure of *mut5-1* and *rad51-1* to complement to restore γ-ray

resistance and efficient sporulation, and the mapping of both markers distal, but closely linked, to *trp 2* on the right arm of linkage group V, leaves little doubt that they are allelic.

The observation that the frequency of UV-induced homozygosis of *ade 2* was unchanged in *mut 5*-bearing strains (Table 20) was not consistent with the observation of Saeki et al (1974) that radiation-induced intergenic recombination was reduced in *rad51-1* homozygotes. When unselected clones from homozygous *mut 5*, heterozygous and wild type diploids were examined for the segregation of recessive markers it was found that the frequency of clones in which recessive markers were uncovered was not affected to any great extent by the presence of *mut 5*. The spontaneous occurrence of apparent non-disjunction products in the *mut 5*-bearing diploids was of considerable interest (Table 25). The investigation which followed was designed to test the reality and the reproducibility of this observation and to attempt to define the cause.

The markedly increased frequency of can R $ura^-mth^-his^-$ clones among the can R isolates of the homozygous *mut 5* diploids compared with the wild types (Table 26) confirmed the initial observation. Incomplete dominance of *mut 5* with regard to this phenotype was apparent in the heterozygotes.

On the basis of the assumption taken (that the aberrant segregants from heterozygotes and *mut 5/mut 5* diploids have identical linkage group V complements), the occurrence of 3 or 4 viable meiotic products in asci obtained by sporulation of aberrant segregants from *mut 5* heterozygotes (Table 27) indicates that, if non-disjunction is the cause, then two such events are necessary. The first is required to eliminate the chromosome bearing the dominant alleles, reducing the chromosome

number to 2n-1, and the second to restore the monosomic chromosome, which carries the recessive alleles, to disomy. An alternative is that in *mut 5* strains coincident spontaneous mitotic exchanges, one on each side of the centromere, involving the same two chromatids, are favoured. Both hypotheses are consistent with the viability data obtained. Strömnaes (1968) found that growth of diploids in the presence of p-fluorophenylalanine resulted in the production of similar aberrant segregants. He, too, was unable to choose between these hypotheses.

Confirmation of the restitution of the monosomic chromosome by a second event may be obtainable by scoring the viability of meiotic products of aberrant segregants shortly after their occurrence, and at intervals thereafter. Early samplings should show high frequencies of 2:2 first division segregations for viability, which should give way to 4 viable:0 inviable spores with continued growth (Mortimer, personal communication).

While it might be difficult to reconcile it with the incomplete dominance of *mut5-1*, the possibility that the aberrant segregation pattern seen in *mut 5* homozygotes was a manifestation of a chromosomal rearrangement was considered. No hypothetical configuration was found which would account for both the aberrant segregations seen in the diploids and the production of 4 viable spores from meiosis in the aberrant segregants, or for that matter, in any segregant which resulted from an exchange within a rearranged segment. No indication of the presence of a rearrangement involving linkage group V was seen in the crosses analyzed in the mapping study.

Parameiosis, the resolution of pre-meiosis I conditions in cells returned to vegetative growth (Simchen et al, 1972), allows meiotic

levels of recombination to occur without the completion of the sporulation process (Sherman and Roman, 1963; Esposito et al, 1974). The results presented in Table 30 and Figure 31 indicate that *mut 5/mut 5* cells shifted back to nutrient medium at 8h after exposure to sporulation medium revert to vegetative growth and exhibit recombination frequencies like the wild type cells. In the wild type, and presumably in the *mut 5* homozygous strains, eight hours exposure to sporulation medium should have brought the cells into the period of pre-meiotic DNA synthesis (Simchen et al, 1972, 1976; Hopper et al, 1974). It is clear from the data that beyond this time the frequency of histidine prototrophs is lower in *mut 5* homozygotes than in the wild type. Whether this reflects a deficiency in the *mut 5* strains in pre-meiotic DNA synthesis or an inability to resolve as efficiently as the wild type, a pre-meiotic condition, even on return to vegetative growth, is not clear.

The decline in relative viability at 44h seen in the wild type and the continued increase in the frequency of histidine prototrophs suggests that cells which have failed to complete meiotic events by this time are losing viability.

The decline in viability of the *mut 5/mut 5* strains at this time is accompanied by a decrease in the frequency of prototrophs. It must, I think, be assumed that most of the histidine prototrophs seen for the *mut 5/mut 5* strains in this experiment are of parameiotic origin. Taken together, the low viability of meiotic products from *mut 5/mut 5* diploids and the depressed frequencies of asci seen here, would predict that the histidine prototrophs derived from completion of meiosis in the *mut 5* homozygotes constitute as little as 10% of those that are observed. On this basis, the decline in the frequency of prototrophs at 44h can be

interpreted to mean that commitment to meiosis in *mut 5/mut 5* cells is to a large extent a lethal event. Whether this is a result of failure to complete DNA synthesis properly or an inability to resolve recombination events, remains to be seen.

The late appearance of asci in the heterozygous strains relative to the wild type may represent another dominant *mut5-1* effect. However, the concentrations of cells in the heterozygote cultures were suboptimal (0.64 and 0.25 x 10^7 cells/ml) for the heterozygotes when harvested from pre-sporulation medium. The concentrations of the *mut 5/mut 5* clones 1 and 2, and the wild type cultures were 1.2, 0.72 and 1.5 x 10^7 cells/ml respectively. A concentration of 1 - 1.5 x 10^7 cells/ml is generally considered optimal. The low titre of the heterozygote suspensions could account for the sporulation delay.

In the description of the results of the UV-induction experiments for *mut* bearing strains, passing reference was made to the variability which is seen in the transition from the lower initial slope to the higher final slope of the mutation and intragenic recombination dose response curves. The data obtained on the induction of histidine prototrophs in homozygous *mut 5* diploids suggests a possibility which may be worth further investigation.

Comparison of Figures 24 and 26 indicates that the initial slope of the *mut 5/mut 5* curve (which is in reality a mutation induction curve) is greater than that of the wild type. Extrapolation of the *mut 5* curves (ignoring for the moment the 3 curves which deviate above 63 J/m^2) brings them relatively close to the wild type curves at the latter's transition region. The possibility that the final phase of the recombinant

induction curve is in reality a mutation induction curve appears to be reasonable.

The 3 *mut 5/mut 5* histidine prototroph induction curves which deviate from the others above 63 J/m^2 provide a partial explanation for the variable transition behaviour of many of the curves obtained in this work. The survival data for the three strains involved indicate, by an inflection in the survival curve, the presence of a resistant subpopulation in all three (see Table 17A, D and G). This could account for a plateau, but it is not entirely clear to me that it can explain a decline.

That the presence of a resistant subpopulation may be but a partial explanation becomes clear when one attempts to correlate the plateaux in induction curves with inflections in survival curves. In Figures 1 and 2, for example, the transition phases of the mutation curves do not correspond in any fixed way to the inflections in the survival curves. As with the induction of recombinants, the possibility that there are two systems involved may be worth further study.

Of the mutators studied, only *mut5-1* can be placed with certainty in an already identified epistasis group. The data indicate however that *mut 10* may also be a component of this same recovery process. While the failure of *mut3-1* and *mut4-1* to show any effect on UV-induced mutation or recombination may be attributable to any one of a number of causes, for example leakiness, the possibility must be considered that they are components of a recovery process that has not been, or cannot be, defined on the basis of interactions of mutations on UV-exposure. This is also true of *mut1-1*, *mut2-1*, *mut6-1* and *mut9-1*.

The existence of other recovery routes besides the 3 defined on the basis of the interactions described earlier is suggested by several observations. The nature of the interactions of mutation in *rad 6* or *rad 18* with one another, and with mutations at other repair loci, that is seen following X-irradiation is not the same as that seen after UV-exposure (discussed in the introduction). Accepting for the moment that the *rad* loci encode repair enzymes, one is led to the conclusion that the processing of different specific DNA lesions may require the function of different combinations of these enzymes.

This conclusion is also suggested by the work of Prakash (1976) on chemical mutagenesis in radiation sensitive mutants. Ethylmethanesulphonate mutagenesis, while it does require function of the *RAD 18* pathway loci, *RAD 6* and *RAD 9*, also requires *RAD 52* function. Mutation of *RAD 22*, a *RAD 3* epistasis group member, results in decreased mutability of nitroquinoline oxide, implicating this gene's function, with those of the *RAD 18* epistasis group loci, in yet another lesion-handling process. Not only the lesion itself, but also the nucleotide sequence in the vicinity of a lesion may determine the "pathway" used (Lawrence and Christensen, 1976).

The identification of many "new" presumptive repair loci using MMS-sensitive mutants (Prakash and Prakash, 1977), which may make confirmation of *MUT 2* and *MUT 9* as DNA repair loci possible, supports the view that there are repair systems which remain to be defined.

This characterization of the mutator loci with regard to parameters that identify components of the DNA repair system in yeast was carried out in the expectation that an understanding of the relationship between them would bring us closer to an understanding of spontaneous mutation. While the precise relationship of most of the mutator loci with repair processes is still not understood, and despite the confusion that surrounds some aspects of these processes, it is still apparent that the only reasonable context in which to consider spontaneous mutation is with regard to the DNA repair system.

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APPENDIX

Location of *RAD 51*, *RAD 4*, and *RAD 3* on Linkage Group V

The data contained in Table 24 make it possible to locate *RAD 51*, *RAD 4* and *RAD 3* fairly precisely on the right arm of linkage group V. Table A1 contains the pooled data from Table 24 for recombination between adjacent markers (see R. K. Mortimer and D. C. Hawthorne, 1973, Genetics 74, p. 47) and between other markers used to define the position of *RAD 3*.

The distances computed for the intervals between adjacent markers are consistent with those indicated by the map of Mortimer and Hawthorne, 1973. *RAD 51*, which is placed distal to *TRP2* on the basis of crosses involving these and other linked markers (see text), is just under 3 centimorgans from *TRP2* on the basis of the pooled data from crosses involving *trp 2 - mut5-1* and *trp 2 - rad51-1*.

While linkage of *rad 4* with *rad 51 (mut 5)* and of *rad 4* with *rad 3* was detected, no linkage of *rad 3* with *rad 51* was apparent. On this basis *rad 3* was placed distal to *rad 4*. The map follows:

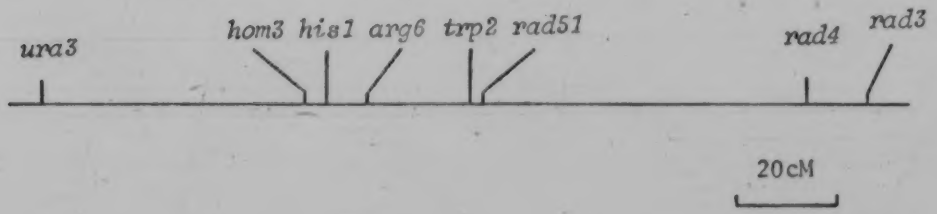

TABLE A1

Pooled genetic mapping data from TABLE 24

Gene pair	Tetrad types PD	NPD	T	Source (Table 24 panel)	Distance* (cM)
ura3-hom3	46	11	131	A, E, G	52.4
hom3-his1	205	0	19	E, G	4.24
his1-arg6	138	0	28	A, F, G	8.43
arg6-trp2	47	0	31	C	19.9
trp2-rad51 (mut5)	338	0	21	C, D	2.92
rad51-rad4 (mut5)	89	28	244	E	57.1
rad4-rad3	71	0	25	F	13.0
rad51-rad3	9	6	47	E	

* Distance = (T + 6NPD) x 100 / 2 x Total (D. D. Perkins, 1949, Genetics 34: 607-626).